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OBSERVATIONS ON DENERVATION OF THE CAROTID SINUSES AND SECTION OF THE DEPRESSOR NERVES AS A METHOD OF PRODUCING ARTERIAL HYPERTENSION

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According to the studies of Hering, Heymans and their collaborators, it is possible to obtain a durable arterial hypertension uncomplicated by destructive lesions of important viscera. By denervating the carotid sinuses and sectioning the depressor nerves in rabbits, Koch and Mies (1929) and Kremrr et al. (1933) obtained an arterial hypertension which they state persisted as high as 170 mm. Hg for two years. Heymans and Bouckaert (1933) reported similar results in dogs following the same type of operation. Dautreband (1934) reported pressures as high as 200 mm. Hg maintained for a like time. In this laboratory, Degroat, Davis and McDonald (preliminary report, 1933) experienced no difficulty in demonstrating the immediate hypertension resulting from denervation of the carotid sinuses and section of the depressor nerves, but the phenomenon appeared to be relatively transient. Likewise transient was the hyperepinephrinemia which had been previously described by Heymans (1931). There were no alterations in the respiratory rate.

In view of the importance of these findings to further experimental investigation of hypertension, it was considered worth while to determine the course of blood pressure changes which occur after complete denervation of the carotid sinuses and aortic sensory areas.

EXPERIMENTAL METHODS. Blood pressure determinations on rabbits were made indirectly in most cases by the method described by McGregor except that the first reading was used instead of those recommended by this author. The method was checked in many instances against pressures taken from a cannula in an artery, using ether anesthesia, and when the readings were not the same, the variation was only a few millimeters.

On dogs, blood pressure was determined by both direct and indirect methods. The direct determinations were made by puncturing the exposed femoral artery with a 24 gauge needle connected with a small caliber mercury manometer. The anesthetic used was ordinarily morphine and novocaine, though occasionally ether was used. The indirect pressure determinations were made by auscultation using a sphygmomanometer cuff 7 cm. wide wrapped around the upper part of the fore-leg. On large dogs this method gives consistent results, the readings being a few millimeters higher than those taken directly.

In the rabbit the sinuses were denervated and the depressor nerves cut, either at the same operation or in two stages. Denervation was accomplished in both rabbits and dogs by stripping away all connective tissue from the carotid bifurcation and along each vessel, painting the surfaces with 85 per cent phenol then washing with alcohol.

In the dog, a separate depressor nerve is described by Ellenberger and Baum, and by Heymans and Bouckaert (1933). The latter authors state that they are able to isolate and section this nerve and this procedure in conjunction with denervation of the sinuses has resulted for them in the production of a permanent hypertension. We have been less fortunate in finding a separate depressor nerve and when a bundle could be separated which answered the above authors' descriptions, we were unable in acute experiments to demonstrate any depressor effects. For this reason we have endeavored to devise an operation which would be more certain in its execution. Attempts to denervate the cardio-aortic depressor areas by cutting the vagus nerves, the right one below the recurrent branch, then followed by denervation of the carotid sinuses, were uniformly unsuccessful because of the occurrence of disturbances of the digestive tract as described by Herzen (1897).

The operation finally adopted permits complete and certain section of the four vaso-sensory nerves, allows indefinite survival of the animal and is almost without mortality. It is performed in three stages: 1, anterior transpleural section of the right cardiac nerves; 2, denervation of the carotid sinuses as described, and 3, section of the left vagus nerve in the neck. Twelve dogs were operated on in this manner with entirely uniform results.

EXPERIMENTAL RESULTS. In a series of 12 rabbits successfully denervated, the immediate and late effects upon blood pressure were determined as set forth in table 1. Blood pressure determinations were made by the direct method in rabbits 10, 11, and 12.

The relative importance of the vaso-depressor action of the cardio-afferent portion of the vagus and of the carotid sinuses in the dog is easily noted in our experimental results. (See table 2.) The rise of pressure that occurs after denervating the sinuses alone is usually less marked than

that which occurs after denervation of the sinuses and section of the vagi. It is also of shorter duration. All of the depressor fibers must be divided

TABLE 1

Showing changes in blood pressure of rabbits immediately after denervation of the carotid sinuses and section of the cardio-aortic nerves and after varying periods of time post-operative

Readings marked "D" indicate that pressures were determined directly; all others were made indirectly.

ANIMAL NUMBER	AGE, MONTHS	PRE-OP.	BLOOD PRESSURES IN MM. Hg	
			Post-op. 2-3 days	Later
1	6	XXX	150	120 (90 days)
2	18	118	130	120 (22 days)
3	11	125	132	120 (28 days)
4	3	118	132	126 (3 days)
5	3	XXX	160	124 (6 days)
6	3	XXX	122	XXX
7	6	XXX	120	XXX
8	3	70	84	XXX
9	3	100	100	XXX
10	6	110	160	D 105 (19 days)
11	6	XXX	174	D 105 (26 days)
12	6	D 103	XXX	D 110 (22 days)

TABLE 2

Showing the effect on blood pressure of denervation of the sinuses alone (cardio-aortic nerves intact) in the dog

Readings marked "I" were made indirectly; all others directly.

ANIMAL NUMBER	BLOOD PRESSURE IN MM. Hg		
	Before denervation	Immed. after	Later
1	XXX	I 158	138 (2 days)
2	127	184	XXX
3	I 128	170	136 (2 days) 128 (25 days)
4	I 120	XXX	159 (23 days)
5	I 140	XXX	138 (28 days)
6	130	182	XXX
7	113	146	XXX
8	I 130	I 156	XXX
9	118	150	XXX

in order to maintain more than a fleeting rise of blood pressure since one vagus alone, if left intact is sufficient to cause the pressure to return rapidly to its normal level.

Within a few days after the first two stages of the operation have been performed the blood pressure is found to be normal. Section of the remaining (left) vagus under either local or general anesthesia is followed by a dramatic rise in arterial pressure and pulse rate. The results observed in nine dogs so operated are recorded in table 3.

The course of the blood pressure following denervation of the carotid sinuses and section of the cardio-aortic afferent nerves in dogs is not altogether constant. Two phenomena were observed in our experiments: first, an early instability of the blood pressure of variable duration with maintenance of a moderately elevated basal level, and second, a tendency for the pressure to approach the normal limits after a variable period of time. Four dogs were selected for a detailed study of the course of the

TABLE 3

Showing changes in pulse rate and blood pressure following complete denervation of the vaso-depressor system

ANIMAL NUMBER	BLOOD PRESSURE IN MM. Hg		PULSE RATE	
	Before denervation	After	Before	After
2	127	204	52	204
3	128	215	78	180
4	120	259	55	240
5	140	242	60	240
6	130	198	150	240
7	113	232	40	210
10	xxx	240	110	216
11	146	176	80	240
12	110	169	40	120

blood pressure following complete denervation, and the two phenomena may be observed in figure 1, made from data collected on these animals.

The marked fluctuations in systolic pressure which occur with even slight emotional or physical excitation are less manifest from the data plotted in figure 1 than during the actual determinations. The figures plotted into the two lines each for animals 2 and 3 represent the maximum and minimum pressures. The high pressures shown in the figure, with the exception of those immediately following denervation were invariably obtained when the animal was moderately excited, or just following a short period of vigorous exercise. The low pressure readings were always obtained when the animal was resting comfortably or under even anesthesia, and are frequently 5 to 10 mm. Hg lower than the majority of readings taken at the same period with the animal at rest. The marked instability in blood pressure which manifested itself in dog 3 disappeared

in less than eight weeks; that which occurred in dog 2 has maintained itself without diminution up to the present time (120 days).

Dog 11 (not included in fig. 1) illustrates nicely the type of fluctuation in blood pressure which is observed for a variable period of time following complete denervation. The pressure upon completing the last operation rose at once to 176 mm. Hg. On the third post-operative day it was found to be 204 mm. Hg, determined by the indirect method with the animal lightly morphinized. After 10 minutes' rest on the table the blood pressure fell to 164 mm. Hg, but rapidly rose again to 230 mm. when the dog was suddenly aroused. After a further rest, the pressure declined progressively to a minimum of 140 mm. Hg. This procedure was repeated

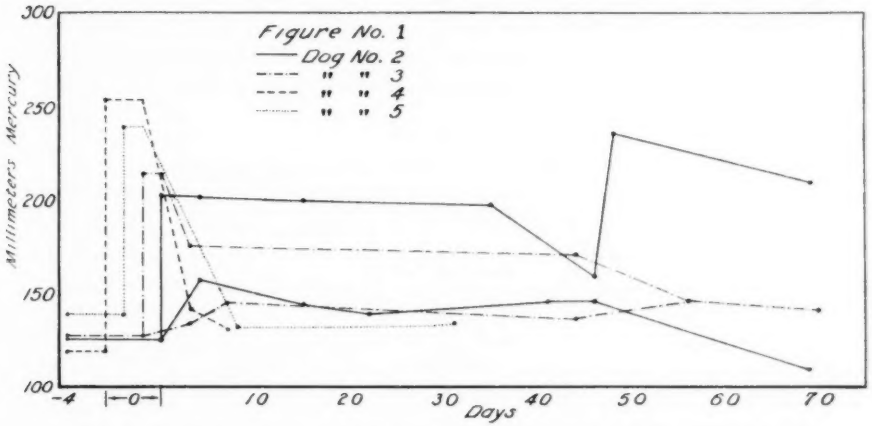

Fig. 1. Showing the variations in systolic blood pressure following complete denervation of the vaso-depressor areas. The two lines of the same type represent maximum and minimum systolic readings on the same animal.

a number of times with the same results. The animal died of distemper five days later.

From the data cited above it is apparent that the basal systolic pressure tends to approach rapidly to the normal. Why the pressures returned so rapidly to a low basal level in dogs 4 and 5 without exhibiting the marked rises when the animal became excited is yet unexplained. The blood pressures during normal activity of dogs with the vasodepressor areas denervated is equally problematical. In some animals, as we have shown in dog 11, the blood pressure appears to remain elevated at all times except when the animal is at complete rest. In such an animal the condition might justly be described as a permanent hypertension. Our experimental results indicate, however, that in other dogs following the denerva-

tion described the blood pressure, initially high and unstable, returns to normal levels after a variable lapse of time.

Various authors have remarked upon the high mortality following denervation of the carotid sinus, many deaths being ascribed to acute cardiac dilatation and edema of the lungs. In rabbits we have observed no deaths which could be attributed to these causes, but in dogs this type of accident appears to have occurred in three animals. Dog 2 suffered for a few days after complete denervation from what the attendant described as fainting spells. This animal, a large collie, appeared to be in excellent physical condition but at feeding time, when jumping about the pen, would fall to the floor and lie quietly for several minutes, then would get up with some indication of weakness. Apparently complete recovery occurred within five minutes. Dog 12, a large airedale apparently in excellent condition, fell to the floor while running about the room. Immediate examination showed the animal to be pulseless and without detectable heart-beat.

TABLE 4

Showing decline of the pulse rate toward normal after complete denervation of the vasodepressor areas

ANIMAL NUMBER	PULSE RATE	
	Immediately post-op.	Later
2	204	116 (106 days)
3	180	86 (41 days)
4	240	140 (3 days)
5	240	120 (14 days)

Gasping respirations continued for about one minute. Attempts to revive the animal were unsuccessful. Autopsy revealed an airless, hyperemic left lung with no inflammatory changes. The condition of the lung was ascribed to the left vagotomy. Dog 4, a large healthy airedale, died suddenly while recovering from ether anesthesia following a direct blood pressure determination seven days after complete denervation. The pulse, taken while the animal was partly recovered from the anesthetic, was 210 per minute; a few moments later breathing suddenly ceased. Autopsy revealed nothing but a dilated right heart. The findings are indicative at least of an acute cardiac dilatation.

The tachycardia appears to us to be chiefly dependent on a loss of vagal tone. This is in agreement with the view of Pierre Herzen (1897). Like the tachycardia of vagotomy, the rate tends to fall progressively. While the initial rate is somewhat higher than that following vagotomy alone, the higher rate may be explained by the response of the heart to the greater load placed upon it. The loss of the inhibition of the cardiac acceleratory

impulses which normally arise reflexly from distention of the carotid sinuses (Bronk et al., 1934) is probably not a factor here because of its short duration. After a variable period of time the pulse rate falls approximately to normal.

Witt, Katz and Kohn (1934) report a high percentage of deaths from respiratory failure during or immediately following denervation of the carotid sinuses and bilateral vagotomy in dogs, cats and rabbits. In the course of our experiments of this nature we have observed this manner of death in no rabbits and in only two dogs, both of which deaths we believe to have been due to over-etherization.

Our observations on the respiratory rates following complete denervation of the depressor systems in dogs were not sufficiently controlled to warrant any definite conclusions. In no case, however, did we observe any changes in respiratory rate except immediately following the actual operation. This is in agreement with Cromer and Ivy (1931) and Gemmill et al. (1933).

SUMMARY

1. The effects of denervation of the carotid sinuses and section of the depressor nerves in a series of twelve rabbits and of denervation of the carotid sinuses and section of the cardiac fibers of the vagus nerve in a series of nine dogs are reported.

2. Following the above cited operation in rabbits we observed a transient elevation of blood pressure, no increase in respiratory rate, and no sudden deaths.

3. The above cited operation in dogs is followed by an unstable blood pressure which tends after a variable period of time to become stabilized at a level not greatly above the normal.

4. Following complete denervation of the depressor system in dogs, sudden deaths may occur from cardiac failure.

5. There follows a tachycardia in dogs which is similar or identical to the tachycardia of vagotomy alone. The pulse rate in rabbits was not followed.

6. After this operation in dogs, there was no increase in respiratory rate.

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DEGREE OF CONSTANCY IN HUMAN BASAL METABOLISM

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In considering whether there is any degree of constancy in the basal metabolism of humans, we are confronted with three problems, namely, how constant is the basal metabolism of an individual from hour to hour on any given day, from day to day over a period of several weeks, and from month to month throughout the year?

The problems center on the question as to whether or not the internal activity of the body unaffected by external factors is constant. Restlessness, immediate after-effect of muscular activity, marked changes in environmental temperature, and other such external factors must be ruled out. But how do the well-known factors, other than digestion and muscular activity, that necessarily enter into every one's daily life affect the basal metabolism? There is a weekly rhythm in the life of practically all individuals. Does this change in the daily routine influence the metabolism? The emotional state likewise plays a rôle and, with women, the menstrual cycle. If effects due to cold and warm weather are certain in man as proven with birds and animals (Benedict and MacLeod, 1929; Benedict and Riddle, 1929), then season should be classified as a factor half way between the exogenous and endogenous factors, which results not in a local cooling or heating effect on some particular day but in acquired reactions to cold and heat. The study is a physiological one, pure and simple, but likewise is of importance in pathology.

Metabolism from hour to hour.[1] The course of the metabolism throughout any given day has been studied frequently, although rarely for this specific purpose. The general conclusion to be derived from a consideration of all the Nutrition Laboratory experience is that with a well-trained subject, accustomed to the experimental requirements, who maintains uniformity in muscular and mental repose and is in the post-absorptive condition, the basal metabolism from hour to hour may be expected to be constant through the greater part of the day, provided that the subject does not sleep or become drowsy or that he is not unduly fatigued by the tests.

[1] For the literature on this topic and the other phases of metabolism treated in this paper see page 530.

Metabolism from day to day. In all physiological laboratories where metabolism measurements are almost a daily routine, numerous observations on the same individual, frequently from day to day but more often on intermittent periods, have been recorded, most of them perhaps with women.

Of the numerous papers published on this question, special attention should be paid to that of Gessler (1925) and of Grollman (1930).

Most of the male subjects showed considerable regularity in the oxygen consumption, while the female subjects in general showed considerable variability, not infrequently directly associated with the menstrual cycle.

Technique used in this research. Of the Nutrition Laboratory's personnel at least two have had their basal metabolism measured from time to time for over 25 years. One of these, F. G. B., was the subject of the experiments here to be reported. His metabolism under basal conditions was determined daily, with four exceptions (Sundays), from May 5 to May 26, 1932, and daily without any exceptions from May 5 to June 6, 1933. In the 1932 series the helmet was used with blower, carbon-dioxide absorbent, and spirometer on the closed-circuit principle, as described elsewhere (Benedict, 1933), and the oxygen consumption was measured graphically from the kymograph tracings of the excursions of the spirometer bell. In the 1933 series the helmet was used on the open-circuit principle (Benedict, 1933) and the measurements included not only the oxygen consumption but the carbon-dioxide production and the respiratory quotient. The helmet, once adjusted, was not removed until the end of the measurements.[2] On each day there were from three to five 10-minute periods. The heart rate was determined by means of a stethoscope attached to the chest. The respiration rate was determined, as usual, from the spirometer tracings.

In the 1933 series on May 10 and thereafter the insensible perspiration was recorded at the end of each morning session by means of a 100 kgm. Sauter balance, installed in the same room in which the subject slept and in which the respiration apparatus was located. All the precautions necessary in such observations were taken. The bedding used on the balance had been exposed to the same atmospheric conditions as had prevailed during the basal metabolism measurements. Care was taken to place on the counterpoise arm of the balance a blanket corresponding to that used by the subject when lying on the suspended bed. The insensible loss in weight was recorded in short periods until uniformity in the rate of loss was established, which usually occurred in a series of three or four 10-minute periods.

Throughout both experimental series the subject lived at the Nutrition

[2] All the observations, including the gas analyses, were made by E. L. Fox.

Laboratory, sleeping in a comfortable bed in a familiar environment. The room temperature varied from 17° to 25° and averaged 22°C. The observations were made in the morning before he arose. Owing to the varied and active life of the subject, the hour of retiring was not the same each night. Rarely did he get to bed before 10 p.m. and frequently it was after 1 a.m. But he had always been lying in bed at least 8 hours before the respiration experiment began and often for 9 or 10 hours. He wore the ordinary cotton night clothing and was suitably covered with light bedding, so that at no time did he feel too warm or too cold.

In the 1933 series precautions were taken to insure against drowsiness. Two small electric lights were placed in the field of vision, one red and the other yellow. These were lighted at will by the operator, unknown to the subject, who was expected to press lightly a telegraph key under his finger, once if the light was yellow and twice if it was red. Both the colored lights and the response of the subject were recorded electrically on a kymograph. This called not only for alertness to designate that the light was lighted but for a slight degree of discrimination to indicate whether the light was red or yellow. This procedure proved satisfactory for a while, but it was soon found that the eyes of the subject were under a strain in watching the lights. Hence these were later supplemented by two electric buzzers of different pitch, suspended near the helmet. Thus when the eyes were closed the subject discriminated between the high and the low pitch buzzer. The graphic record shows that from 30 to 40 signals were given during each experimental period (circa 10 minutes). Signals were rarely missed, though occasionally the subject mistook the high tone for the low tone.

Throughout both experimental series the subject followed in general his usual and varied food habits, both as regards quality and quantity. There was the weekly change in meal hours commonly occurring on Sunday, when the heaviest meal of the day was eaten at noon rather than at night. No conscious adjustment of the diet was made at any time, save perhaps on one or two occasions when an unusually hearty meal was eaten on a Sunday noon, on which occasions the supper at night contained somewhat fewer calories than would otherwise have been eaten. One injunction only was made with regard to eating, namely, that no food was to be taken after 7 p.m. This injunction was carefully adhered to in both series of measurements, except on those occasions when dinner functions called for a somewhat later meal. Following these instances the metabolism measurements the next day were made somewhat later in the morning, to insure that the subject had been at least 12 hours without food.

Each morning, at the conclusion of the respiration experiment but prior to any inspection of results, the subject dictated notes regarding the general character and approximate amount of the last meal, the time of

going to bed, the depth and length of sleep, time of waking in the morning, and subjective impressions during that morning's experiment. In fact, every factor that conceivably might affect the metabolism was taken into consideration and commented upon.

DISCUSSION OF RESULTS. The results of the 1932 and 1933 series of measurements are recorded in tables 1 and 2, respectively. The state-

TABLE 1

Daily variations in basal metabolism of F. G. B.—1932

DATE	APPROXIMATE LENGTH OF NIGHT'S SLEEP	DEPTH OF SLEEP*	BODY WEIGHT** (NUDE)	MOUTH TEMPERATURE	AVERAGE PER MINUTE		
					Pulse rate	Respiration rate	Oxygen consumption
1932	*hours*		*kgm.*	*°C.*			*cc.*
May 5	9	+++	78.5		51	15	236
6	7½	+++	78.0	36.1	52	16	232
7	7	+++	78.3	36.3	48	15	231
9†	?	+		36.3	50	16	233
10	9	++++	79.0	36.2	50	15	229
11	6½	++++	78.0	36.2	51	15	233
12	7	+++	78.0	36.3	50	14	230
13	7	+++	78.0	36.2	53	16	234
14	8	++++	78.5	36.1	53	14	233
16†	6	++++	77.5	36.3	52	15	232
17	7¼	++++		36.5	50	16	235
18	5½	++		36.3	52	15	234
19	3½	+	77.8	36.3	51	15	228
20	6	+		36.2	50	15	216‡
23†	?	++++		36.2	47	16	223
24	5	++	77.8	36.3	51	15	242
25	6	+++	78.5	36.3	53	16	237
26	8	++++	78.5	36.1	50	17	230

* Based on subjective impression. Very well, ++++. Reasonably well, +++. Not well, ++. Very poorly, +.

** Age 61½ years. Height 183 cm.

† Monday.

‡ Drowsy during measurements.

ments in the second and third columns with regard to length and depth of sleep represent personal impressions and are therefore only approximate, although fairly indicative of the relative differences in sleep from night to night. On some occasions he slept undisturbed throughout the night. On other occasions the length of sleep was short and there were a few nights when the actual time of sleeping was in all probability as short as two or three hours. There was no correlation between the length or the intensity of sleep and the body temperature, pulse and respiration rates,

TABLE 2

Daily variations in basal metabolism of F. G. B.—1933

DATE	APPROXIMATE LENGTH OF NIGHT'S SLEEP	DEPTH OF SLEEP*	BODY WEIGHT (NUDE)	BODY TEMPERATURES		INSENSIBLE PERSPIRATION PER HOUR (AVERAGE)	RESPIRATORY QUOTIENT (AVERAGE)	AVERAGE PER MINUTE		
				Mouth	Rectal			Pulse rate	Respiration rate	Oxygen consumption
1933	*hours*		*kgm.*	*°C.*	*°C.*	*gm.*				*cc.*
May 5	5 or 6	++	77.1	35.8			0.78	51	15	233
6	6	+++	76.7	35.9				51	15	230
7	7½	++++		36.0				48	14	221
8**	6½	++++	77.7	35.9			0.88†	50	15	227
9	6½	++++	77.8	35.9			0.83	50	15	234
10	9	++++	76.7	35.9		32	0.80	48	16	232
11	5	+++	76.7	35.9		36	0.80	48	15	229
12	?	+++	77.0	35.8		36	0.82	48	15	227
13	8½	++++	76.4	36.0		35	0.81	50	16	233
14	3 or 4	++	76.9	35.9		39	0.80	48	16	222
15**	7	+++	77.8	35.9		35	0.85†	49	16	225
16	5½	++	77.5	36.1	36.2	34	0.82	50	15	231
17	6½	++++	77.2	36.1	36.2	32	0.80	48	16	236
18	6	++++	76.7	35.9	36.2	31	0.79	48	16	236
19	7½	+++	76.9	36.1	36.2	32	0.79	49	15	236
20	8¼	+++	76.6	36.1	36.3	32	0.78	49	15	233
21	7½	+++	76.2	36.4	36.4		0.85†	52	16	229
22**	6	+++	76.7	36.5	36.4	35	0.84	55	16	248‡
23	7	++	76.5	36.7	36.6	35	0 81	54	17	255‡
24	8	++++	76.4	36.3	36.4	35	0.81	51	15	246‡
25	7	+++	76.4	36.4	36.4	36	0.79	53	15	241‡
26	7	+++	76.2	36.4	36.4	35	0.80	50	16	230
27	6	+++	76.0	36.3	36.3	33	0.81	51	16	232
28	6	+	76.1	36.1	36.2	29	0.78	48	15	233
29**	6½	+++	76.0	36.1	36.1	31	0.80	48	15	229
30	6	+++		35.9	36.2	34	0.79	50	16	237
31	5½	+++	75.7	36.0	36.2	34	0.85	49	16	224
June 1	7	+++	76.6	36.0	36.2		0.84	51	16	226
2	?	+	76.6	35.9	36.1	32	0.83	48	16	222
3	7½	+++	76.5	36.1	36.1	35	0.82	51	16	232
4	7½	+++	76.2	36.1	36.1	33	0.79	49	15	234
5**	5¾	++	76.0	36.0	36.2	34	0.83†	50	16	225
6	7¼	++++	76.8	35.9	36.2	35	0.85	49	16	224

* Based on subjective impression. Very well, ++++. Reasonably well, +++. Not well, ++. Very poorly, ∓.

** Monday.

† Dinner on day before rich in carbohydrate, but not excessively so.

‡ Emotional disturbance.

and the oxygen consumption. The work engaged in by the subject was for the most part intellectual and usually resulted in moderate fatigue at the end of the day. But eight hours of repose in bed, irrespective of actual time asleep, apparently sufficed for complete mental and physical recuperation. In tables 1 and 2 is given a picture of the day to day physiology, which shows what may be expected in the line of variations of the several factors under strictly controlled conditions.

When the day or the night before the experiment was cool, this lower temperature was not reflected in any sense in the metabolism.

Body weights were taken each morning after the bladder was emptied, on a balance sensitive to 0.1 kgm. No apparent reason for the fluctuations in the body weights is at hand, except that the high weights seem occasionally to be coincident with a fairly carbohydrate-rich diet on the day before. Obviously these fluctuations are attributable wholly to changes in water content of the body.

The mouth temperature underwent only slight changes from day to day. It is probably mere coincidence that the highest mouth temperature, 36.7°C. on May 23, 1933, was found with the highest oxygen consumption. The rectal temperatures, recorded only in 1933, were usually one or two tenths of a degree above the mouth temperatures, although in a few instances they were the same or lower. This is the normal picture in such comparisons. On the average the rectal temperature was 36.3°C.

The pulse rate (recorded as the average of 10 or more counts during the oxygen measurements) was fairly uniform throughout each series. In 1932 it ranged from 47 to 53. In 1933 it was in general lower, many rates under 50 being noted. The lowest rate was 48, on ten days. The highest rates, 53 to 55, occurred on May 22 to 25, 1933, and were attributable to an emotional disturbance (see p. 527). On the whole the pulse rates in both series were fairly low, averaging (with the exception of the rates on May 22 to May 25, 1933) 51 and 49, respectively. The highest pulse rates do not invariably accompany the highest oxygen consumption or the lowest rates the lowest oxygen consumption. In both series the respiration rate varied only from 14 to 17, and averaged 15 and 16.

A new factor studied in the 1933 series was the respiratory quotient. The values for the respiratory quotient given in table 2 represent in each case the average of four closely agreeing determinations. The average respiratory quotients range from 0.78 to 0.88 and the grand average is 0.81. The few instances of relatively high quotients, which occur chiefly on Mondays, seem to be correlated with the ingestion of a fairly carbohydrate-rich diet the day before. This diet, however, was not reflected in an increased oxygen consumption, so the respiratory quotient seems wholly independent of the oxygen consumption itself. In the hundred or more determinations made there were no aberrant quotients such as are frequently reported in the literature.

Another factor studied only in the 1933 series was the insensible perspiration. The values range from 29 to 39 grams per hour and the average is 34 grams. These values represent on each day the average of several 10- or 15-minute periods of weighing on the Sauter balance. But since they were the last observations of the morning and the other metabolism measurements had already occupied considerable time, the subject not infrequently became somewhat restless or anxious to terminate the measurements on account of the pressure of office work. The slight irregularity noted in the insensible perspiration measurements may possibly be in part explained by this mental state of the subject. Physically he maintained complete muscular repose during the observations.

The oxygen consumption in the 18 days of observation in 1932 ranged from 216 cc. on May 20 to 242 cc. on May 24. On May 20 the subject commented at the close of the experiment that he was very drowsy and had had more difficulty in keeping awake than in any of the previous experiments. This drowsiness probably accounts for the low metabolism on this day. On May 24 the subject was mentally extremely alert, making plans for the summer with some anxiety as to whether the plans could be consummated, and there was definite tension throughout the entire morning. Since this is the only day in the 1932 series when anxiety occurred and the highest oxygen consumption was noted on this day, it is possible that this mental state played a rôle in increasing the metabolism. On the evening preceding the experiment of May 25 the subject had had an unusually heavy meal. The next to the highest value for oxygen consumption (237 cc.) was found on this morning. This, however, is only slightly above the average oxygen consumption during the 1932 series and apparently the large meal did not produce a significantly higher metabolism. Aside from the metabolism on May 20 and May 24, the range in the oxygen consumption was from 223 to 237 cc., and the average deviation from the average of 232 cc. was $\pm$ 1 per cent.

The minimum oxygen consumption in 1933 was 221 cc. on May 7 and the maximum was 255 cc. on May 23. For four days, from May 22 to 25, inclusive, the metabolism was higher than usual, ascribable to the fact that the subject was under a very considerable emotional strain due to the effects and after-effects of an automobile accident experienced by a member of his family. This accident caused him considerable anxiety, which was only slowly dispelled, and it was not until May 26 that the metabolism returned to approximately the normal level. If these four days are excluded, the range in oxygen consumption during the 1933 series is from 221 to 237 cc. The average oxygen consumption in 1933 was 230 cc. Although the body weight was, on the average, 1.5 kgm. lower in 1933 than in 1932, the average oxygen consumption (with the exceptions noted above) was at essentially the same level in both series. The endogenous

metabolism from day to day was therefore remarkably uniform, but could be profoundly influenced by emotional disturbance.

The results obtained on Mondays, which have been specially indicated in the tables in the date columns, are of interest because of the change in the daily routine on the day before, when the dinner was eaten at noon instead of at night and sometimes was heavier than usual. But otherwise there was no considerable difference in the daily habits, for the subject usually worked in the Nutrition Laboratory for at least half a day on Sunday. Inspection of the oxygen values on the several Mondays when the metabolism was measured shows that the change in the daily routine had no effect on this factor, although, as previously pointed out, it did influence the respiratory quotient.

The average oxygen consumption of 230 cc. per minute corresponds to a 24-hour heat production of 1598 calories. The average insensible perspiration was 34 grams per hour, which implies, in accordance with the predictions of Benedict and Root (1926), a heat production of 1530 calories per 24 hours. This comparison indicates that with this subject the heat production predicted from the insensible perspiration agrees reasonably well (−4 per cent) with the actually measured heat production.

The difference between the uniformity in the metabolism of men from day to day over a period of a month and the lack of uniformity in the day to day metabolism of women during the same length of time may probably be explained in part by the emotional factor. In the literature the effect of the emotions on the metabolism is not infrequently mentioned. With our well-conditioned subject, Miss W., the only disturbing factor explaining her irregularity in metabolism was, so far as we are aware, the menstrual cycle. But the findings in this present study suggest that the high intermenstrual peaks noted in the curve (Benedict and Finn, 1928) for oxygen consumption of Miss W. over a period of two months might perhaps have been explained by some emotional disturbance, had we questioned her closely as to her daily life. The effect of emotion on the metabolism should obviously be borne in mind in the determination and interpretation of basal metabolism observations in the hospital. An emotional strain such as is common with hospital patients increases the metabolism. Is this an integral factor in hospital and diagnostic observations? If so, the basal metabolism measurements made by Boothby and his associates on "hospital normals," however unsatisfactory they may be for purely physiological standards, have their peculiar value for hospital uses.

The main conclusion to be drawn from this study is that under ordinary conditions the basal metabolism of a well-conditioned man may be assumed to be constant from day to day. But it should be recognized that the oxygen consumption per minute may vary as much as 10 to 15 cc. and still be within normal limits. The fact that the metabolism is in

general constant from day to day does not, however, justify the practice so frequent with some investigators of using throughout an experimental series of observations one basal determination for comparison with measurements obtained under conditions other than basal. Nor does it justify the use of a basal value determined on one day for comparison with measurements obtained on some following day when the effect of some factor superimposed upon the basal metabolism is studied. With women particularly this practice is open to criticism.

Influence of season. The fact that there is reasonable constancy in the basal metabolism of humans from hour to hour and from day to day over a period of about one month is apparently well established, but the degree of variability with change in season needs further study. Hitchcock and Wardwell found the lowest metabolism in winter and spring and the highest in summer and autumn. In this they confirm the observations of Benedict and Gustafson. Gessler, on the contrary, found the highest metabolism (total heat production) in January and the lowest in June and July. If his results are calculated per kilogram of body weight, the seasonal variation is even greater than shown by his curve for total heat production. This variability Gessler ascribes to the influence of external temperature. From an analysis of the metabolism data obtained from day to day with F. G. B. it is evident that only a well-conditioned subject can be used for the study of the seasonal effect. With women it is natural to suppose that the influence of menstruation should be taken into consideration in interpreting any data dealing with the problem of season. Indeed it would seem to be out of the question to attempt to establish a seasonal variation of any magnitude with women, although obviously if the study is based on a *group* of women, as was done by Benedict and Gustafson, individual variations would be more or less equalized. There still remains the problem as to what would be the normal variations of basal metabolism in well-conditioned women after the climacteric.

SUMMARY

The metabolism of a man was measured under basal conditions with the helmet respiration apparatus on both the closed- and the open-circuit principles on eighteen consecutive days (with four omissions) in May, 1932 and on thirty-three consecutive days in May and June, 1933. The results indicate that with a well-conditioned man in complete muscular and mental repose and at least 12 hours after food, the measured physiological functions, body temperature, pulse and respiration rates, respiratory quotient, oxygen consumption, and insensible perspiration, were extraordinarily uniform from day to day and were not affected by variations in the length and depth of sleep, the normal fluctuations in room temperature, or rather considerable variations in the character and amount of the meal

the evening before. The ingestion of a fairly carbohydrate-rich meal at noon resulted in a somewhat higher respiratory quotient the next morning, but did not alter the oxygen consumption. The insensible perspiration was closely correlated with the basal heat production. An emotional disturbance caused a marked increase in metabolism, which did not subside for several days.

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A STUDY OF THE OXIDATION THAT OCCURS IN THE DOG AFTER THE INGESTION OF GALACTOSE

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Recent reports have raised questions regarding the metabolism of galactose which, it seemed, might be favorably investigated by a study of the respiratory metabolism after the administration of this carbohydrate. Roe and Schwartzman (1) found that following the ingestion of galactose there was no greater galactosemia, and only slightly greater glucosemia, in diabetic patients than was observed in normal subjects. These authorsalso found that insulin does not influence the rate of removal of galactose from the blood stream of rabbits. Kosterlitz and Wedler (2) observed that galactose, when fed to diabetics, produces less glycemia and glycosuria, and has a greater antiketogenic effect, than glucose when administered in corresponding amounts. Karr and Letonoff (3) made respiratory quotient observations upon a diabetic patient receiving galactose by mouth which indicated that galactose is not oxidized by the diabetic in any greater quantities than other ingested carbohydrates. These reports suggested that it would be of interest to study the effect of the ingestion of galactose upon the respiratory quotient of the normal and the depancreatized dog. In this paper we are reporting the results of such a study.

EXPERIMENTAL PROCEDURE. The respiratory metabolism of the dogs was determined by both the closed-circuit and the open-circuit methods.

For the closed-circuit studies a Benedict universal apparatus was used in connection with a respiration chamber having a capacity of 250 liters. The chamber is double-walled, and insulated by a large water jacket. The rate of air flow through the chamber is over 50 liters per minute. The accuracy of this apparatus, determined at intervals by alcohol checks, was found to be within 2 per cent without residual analyses. Oxygen was fed by hand from a small cylinder and measured by weighing the cylinder to

[1] The data reported in this paper are taken from a dissertation presented by Joseph H. Roe in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Yale University, 1934.

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±20 mgm. before and after each metabolic period. This apparatus was used for determinations upon both normal and depancreatized dogs.

The open-circuit studies were made by the use of a Douglas bag and a Haldane-Bailey gas analysis apparatus. Twelve-minute expiratory air samples were collected in the Douglas bag with the aid of a respiratory mask, such as used by Boyd, Hines and Leese (4). A dog was thoroughly trained to lie still and breathe normally during the collection of the expired air. The procedure followed was to place the mask over the dog's nose and by means of a three-way stopcock between the mask and the Douglas bag the expired air for approximately 5 minutes was discarded. A period for discarding expired air, previous to collecting a sample for analysis, was allowed in order to permit the dog to become adjusted to the mask and to wash out the atmospheric air from the mask and the tube connecting it to the Douglas bag. After the preparatory period the stopcock was turned and expired air was collected in the Douglas bag for 12 minutes. A sample of the air collected was introduced immediately into the gas analysis apparatus and a second sample of 200 to 300 cc. was placed in a storage flask over calcium chloride solution, sp. gr. 1.40, to be analyzed later as a check. One analysis was thus performed as soon as the sample had been collected and the duplicate analyses were made within 2 to 5 hours after collection of the sample. The Douglas bag used was checked and found to lose 0.033 per cent of CO_2 per hour. As samples of gas were taken for analysis and storage immediately after the end of the collection period, it is obvious that there was no error of significance due to leaking of CO_2 from the Douglas bag. The gas analysis apparatus was checked against outside air and sufficient skill in the manipulation of this apparatus was acquired before experiments were begun. This apparatus was used for determinations upon normal dogs.

The normal dogs used in these experiments were fasted in most cases for 48 hours in order to deplete the glycogen stores of the body before observations were made. In a few instances, where it was desired to start an experiment with the respiratory quotient at a higher level, the animals were fed extra carbohydrate on the day before, but in all cases the animals were fasted for not less than 15 hours before an experiment was carried out.

For the pancreatectomy studies the dogs were totally depancreatized under nembutal anesthesia. During the second and third days after operation the animals were given small doses of insulin, and glucose was administered by mouth, or subcutaneously, in amounts sufficient to protect against hypoglycemia. The animals were then placed upon a diet of raw meat, sucrose, and raw pancreas, and insulin was administered in amounts sufficient to keep the urine practically sugar-free. When a metabolic test was to be carried out, insulin was withheld for 48 hours and no food was allowed for 24 hours previous to the test.

RESULTS. When 2 grams of galactose per kilogram of body weight were administered by mouth to a normal dog and metabolism determinations were made at approximately hourly intervals, only a very slight rise in the respiratory quotient was observed during periods of 3 or 4 hours following ingestion. Data of a representative experiment with this dosage of galactose are shown in table 1.

TABLE 1

Respiratory quotient and blood galactose after galactose ingestion

Female dog, weight 12.7 kgm. Dose, 2 grams per kgm. Fasted 48 hours. Open-circuit method

MINUTES AFTER INGESTION	PROCEDURE	R.Q.	BLOOD GALACTOSE, MGM. PER 100 CC. OF BLOOD
	Basal	0.70	
	26 grams galactose by mouth in 200 cc. H_2O		
48–60	First air sample	0.71	203
112–124	Second air sample	0.75	264
185–197	Third air sample	0.74	132
245–257	Fourth air sample	0.74	66

Urinary excretion of galactose in 24 hours —14.07 grams, or 54 per cent of amount ingested.

TABLE 2

Respiratory quotient and blood galactose after galactose ingestion

Female dog, weight 12.7 kgm. Dose, 5 grams per kgm. Fasted 15 hours. Open-circuit method

MINUTES AFTER INGESTION	PROCEDURE	R.Q.	BLOOD GALACTOSE, MGM. PER 100 CC. OF BLOOD
	Basal	0.76	
	65 grams galactose by mouth in 200 cc. H_2O		
49–61	First air sample	0.81	363
117–129	Second air sample	0.92	551
185–197	Third air sample	0.86	379
241–253	Fourth air sample	0.81	250

When 5 grams of galactose per kilogram of body weight were administered by mouth to a normal dog, the respiratory quotient was elevated to an extent characteristic of carbohydrate oxidation. Representative results obtained with this dosage of galactose are shown by the data of table 2.

A summary of our respiratory quotient experiments with 2 grams and 5 grams per kgm. doses of galactose is shown graphically in figure 1. In

figure 2 is shown a comparison of the responses obtained, when galactose, fructose, and glucose, in doses of 2 grams per kgm. of body weight, were administered by mouth to the same dog under similar experimental conditions.

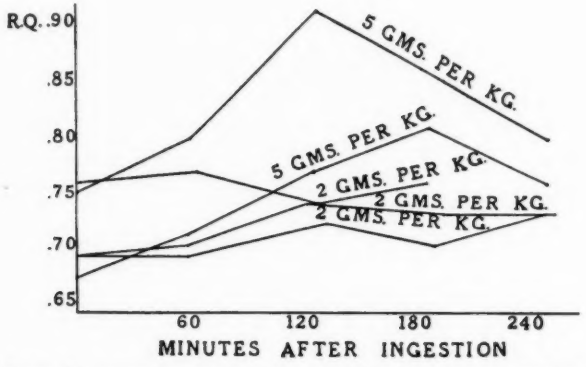

Fig. 1. A graphic summary of respiratory quotient observations following the oral administration of 2 grams and 5 grams per kgm. doses of galactose to a normal dog.

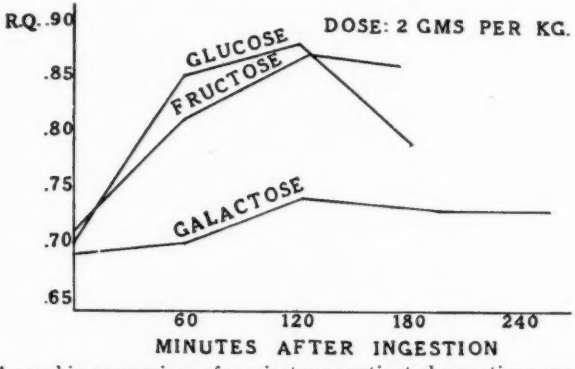

Fig. 2. A graphic comparison of respiratory quotient observations upon a normal dog following the ingestion of glucose, fructose, and galactose, in 2 grams per kgm. doses under identical experimental conditions.

An experiment in which 10 grams of galactose per kilogram of body weight were administered by mouth to a normal dog resulted in an elevation of the respiratory quotient to values as high as 1.

The administration of galactose to depancreatized dogs did not produce an elevation of the respiratory quotient, both small and large doses being

used. The results of 3 experiments with depancreatized dogs in which large doses of galactose were fed, are shown graphically in figure 3.

To remove any doubt concerning the absorption of galactose, samples of blood were collected in the experiments with the open-circuit apparatus immediately after the collection of the expired air samples and were analyzed for galactose by the method of Roe and Schwartzman (1). The presence of galactose in the blood in considerable amounts in the experiments with normal dogs was thus demonstrated. The quantity of galactose in the 24 hour urine samples following galactose ingestion was also determined in several experiments. No question can therefore be raised regarding absorption of the ingested galactose in the experiments with normal dogs, and it is reasonable to assume that adequate absorption occurred in the studies with the depancreatized animals, since we have carried out

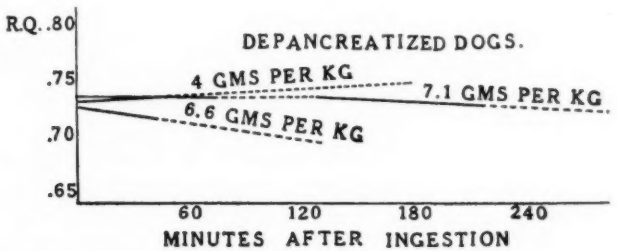

Fig. 3. A graphic representation of respiratory quotient observations upon 3 depancreatized dogs following the oral administration of galactose. Closed-circuit apparatus was used. The solid lines represent periods of equilibration; the broken lines indicate periods of observation.

other experiments with depancreatized dogs in which no interference with absorption occurred.

The failure of galactose to increase appreciably the respiratory quotient, when doses of 2 grams per kgm. of body weight were administered, is of significance. These data seem to show that galactose per se is not oxidized in the dog. Certainly, when the blood galactose is as high as 200 mgm. per 100 cc., there should be a more marked elevation of the respiratory quotient than was obtained in these experiments if galactose is oxidized. This assumption is also supported by the fact that there was a delay in the elevation of the respiratory quotient when large doses of galactose were administered, as shown in figure 1 in which there is only a slight elevation of the respiratory quotient curve at the time of the analysis one hour after ingestion.

The final elevation of the respiratory quotient by large doses of galactose is probably due to the conversion of galactose into a form of carbohydrate

that is readily oxidized in the dog. This hypothesis is consistent with the fact that the dog has a very low renal threshold and excretes a large amount of unchanged galactose in the urine. In the experiments with the small doses the amount of oxidizable carbohydrate that was formed from galactose was too small to produce appreciable elevations of the respiratory quotient. In the experiment of table 1 the urinary galactose was 54 per cent of the amount ingested. Thus, when 2 grams per kgm. were administered, 26 grams of galactose were made available to the tissues, but 14 grams were excreted in the urine and only 12 grams were retained. This amount is not large and, if converted to oxidizable carbohydrate, the change took place over a period of 3 or 4 hours. As the dog had been fasted it must be assumed that some of the oxidizable carbohydrate might be stored as glycogen. An appreciable elevation of the respiratory quotient need not be expected when 12 grams of oxidizable carbohydrate are made available to the tissues of a fasting dog over a period of 3 or 4 hours. With the administration of larger doses of galactose, however, the amount retained becomes more significant, and hence, if conversion to oxidizable carbohydrate does occur, a greater elevation of the respiratory quotient would be expected.

Evidence will be presented in another paper to show that the oxidizable carbohydrate formed from galactose in the animal body is glucose. This assumption is in accord with the observations in which there was essentially no change in the respiratory quotient of the depancreatized dogs following galactose ingestion. Insulin is essential to stimulate the oxidation of glucose; hence, if galactose is converted into glucose in the depancreatized animal, there is no reason to expect an elevation of the respiratory quotient, because insulin is not present.

The suggestion that galactose is not oxidized as such in the dog has never been made in the literature. The data of our experiments and of those in the literature are in agreement, however. Lusk (5), in 1915, determined the respiratory metabolism of a normal dog following the ingestion of galactose. The dog used by Lusk weighed approximately 12 kgm. and the amount of galactose administered was 50 grams, thus making the dosage about 4 grams per kgm. of body weight. Lusk observed a respiratory quotient of 0.93. In one of our experiments, in which a dosage of 5 grams per kgm. of body weight was used, the respiratory quotient rose from 0.76 to 0.92 when the dog had not been fasted severely; and in another experiment, in which the dog had been fasted for 72 hours, the elevation of the quotient was almost as marked.

The only other report in the literature of respiratory quotient studies upon dogs following galactose administration is that of Wierzuchowski (6). In Wierzuchowski's experiment 2 grams per kgm. of body weight were injected per hour for 3 hours, making a total administration of 123.6

grams to a 20.6 kgm. dog. With this large dose a rise in the respiratory quotient was obtained. The response was slow at first in Wierzuchowski's experiment, and the greatest elevation of the quotient observed was 0.88. These results are entirely in agreement with our data, though Wierzuchowski did not place the same interpretation upon them.

Respiratory quotient studies following the ingestion of galactose by human subjects have been reported by Deuel (7), Cathcart and Markowitz (8), and Carpenter and Lee (9). These workers observed marked elevations of the respiratory quotient during the first hour after the ingestion of approximately 1 gram of galactose per kgm. of body weight. Carpenter and Lee obtained some elevation of the respiratory quotient within 15 to 30 minutes after ingestion and also observed a rise in the quotient from the ingestion of as little as 5 grams of galactose. The effect of galactose ingestion upon the respiratory quotient of human subjects thus differs fundamentally from that observed with dogs.

To check our results with dogs against those obtained by other workers with human subjects, experiments were carried out upon a human subject. This seemed important because differences in the purity of the sugar, or in experimental procedure, might be sufficient to produce different results. Forty grams of galactose were given by mouth to a human subject under fasting conditions and respiratory quotient determinations were made by the open-circuit method. The basal quotient was 0.76 and that obtained for the air sample collected 45 to 57 minutes after ingestion was 0.89. This response is of the same magnitude as that obtained by Carpenter and Lee with their human subject upon the same dosage of galactose. Experiments of this type thus served to bring our data upon dogs into relation with the results obtained by other workers who used human subjects.

A species difference in response to galactose ingestion is thus indicated from our observations and from the data in the literature. The difference between man and the dog is perhaps not a fundamental dissimilarity in cellular metabolism, and probably can be explained by the different response of the liver and kidneys of these species to galactose ingestion, which in turn may be due to a difference in adaptation as a result of the greater galactose content of the natural diet of man. Harding and Van Nostrand (10) fed 50 gram doses of galactose to 14 normal fasting human subjects and determined the total urinary galactose excretion. The greatest excretion observed was 13.6 per cent and the average for the 14 subjects was 4.1 per cent. When normal fasting dogs are fed galactose in comparable amounts, the urinary galactose excretion is much greater. In our respiratory metabolism experiments in which the dog was given 2 grams and 5 grams of galactose per kilogram of body weight, the urinary excretions were 54 and 63 per cent of the ingested galactose, respectively. Thus, it is seen that the human subject retains galactose much better than

the dog. This is probably because the liver of the human subject converts galactose into glucose more rapidly than does the liver of the dog, a response which would account for the more rapid and more marked elevation of the respiratory quotient, and the smaller renal losses, after galactose ingestion by the human species.

The low renal threshold for galactose in dogs cannot be considered an explanation of the apparent non-oxidation of this substance. Even though the dog excretes a large amount of galactose in the urine, it must be realized that a large part of the amount ingested comes into intimate contact with the tissues in which carbohydrate oxidation takes place, as indicated by the fact that the blood contains rather large amounts of galactose for several hours after ingestion. The theory of the non-oxidation of galactose in the dog thus loses no support because of the marked renal excretion of this sugar.

SUMMARY

1. The respiratory quotient of normal fasting dogs was not elevated significantly by the ingestion of 2 grams of galactose per kilogram of body weight. When 5 grams, or more, of galactose per kilogram of body weight were administered by mouth, there was a delayed elevation of the respiratory quotient which was characteristic of carbohydrate oxidation.

2. There was no significant change in the respiratory quotient of depancreatized dogs following the ingestion of either small or large doses of galactose.

3. Based upon these observations, it is suggested that galactose is not oxidized as such in the dog. The significant elevation of the respiratory quotient following the ingestion of galactose is attributed to the oxidation of glucose, a plethora of which is produced in the dog by the conversion of galactose into glucose.

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THE GASEOUS METABOLISM OF FROG KIDNEY

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Although considerable work has been done on the gaseous metabolism of the mammalian kidney, little information seems to be available concerning the respiratory processes of amphibian kidney. Such information is desirable since the amphibian kidney has been used extensively in experiments on the theory of renal function. In this paper we furnish data on the respiratory quotient of frog kidney and the effect both upon the respiratory quotient and the oxygen consumption of the addition of glucose, phlorhizin, neutral red and phenol red to the Ringer solution bathing the renal tissue.

METHOD. After removal of adherent tissue the kidneys, which had been dissected from medium sized frogs (R. pipiens), were cut into cubes or slices about one millimeter thick. The oxygen consumption was determined both with the alkali-inset method and with Warburg's "Kästchen," CO_2—bicarbonate method. With the alkali inset method the vessels were also provided with a side arm for tipping substances onto the tissue. Measurements were made manometrically according to the Warburg technique and volumetrically according to the Thunberg technique. In the experiments with the "Kästchen" CO_2 bicarbonate method, Ringer solution containing 0.285 per cent bicarbonate was used according to the formula of Hamburger and Brinkmann (1918); this solution was equilibrated with a gas mixture containing 5 per cent CO_2 and 95 per cent O_2.

The R.Q. was determined according to the method of Meyerhof and Schmitt (1929) using vessels of the type shown in figure 1. We have found this a very convenient all-purpose vessel and considerably more satisfactory than the double sac vessels described by Meyerhof and Schmitt (1929). The barium hydroxide is placed in the side inset deep within the vessel, a position which greatly reduces the likelihood of CO_2 contamination from the air during the preparation. The acid is placed in the ground joint side arm. This procedure eliminates the danger of premature mixing of acid and alkali, a serious hazard with the double sac technique. After the preliminary determination of the O_2 consumption, the acid is tipped onto the tissue in Ringer solution in the main section of the vessel and the resultant solution is thoroughly mixed with the barium hydroxide in the

inset. The vessel is so constructed that when the proper amount of Ringer solution is used in the main portion of the vessel the latter operation is easily possible and premature mixing of Ringer solution and alkali never occurs. The vessel may be used also with KOH in the inset as an ordinary vessel for measuring oxygen consumption either with the ground joint side arm for tipping substances onto the tissue in the main chamber or merely with a ground joint stopper. The latter convenience is made possible by the use of interchangeable ground joints.

With the exception of some of the experiments on R.Q., the work was done on summer frogs. Phosphate Ringer solution consists of one part of isotonic sodium phosphate buffer, pH = 7.6, plus nine parts of Ringer solution.

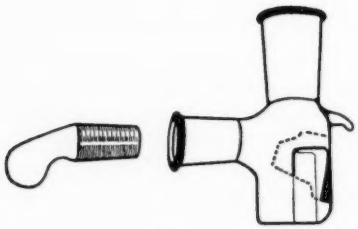

Fig. 1

RESULTS. *1. Oxygen consumption.* The respiration of excised frog kidney is subject to great variation not only from season to season and from one shipment of frogs to the next but also among frogs of a single shipment. Representative data obtained from experiments made chiefly on summer frogs over a period of three years are presented in tables 1 to 4. The Q_{O_2} of kidney in plain Ringer solution averages 1.80 (1.25 to 2.31), in Ringer-bicarbonate (Kästchen method), 2.20 (1.31 to 2.99), in phosphate Ringer, 2.50 (1.24 to 3.64). Although it is the rule that phosphate accelerates oxidative processes in excised tissues generally, the increase observed in these experiments seems exceptionally high. These findings are in line, however, with those of Irving (1928) who found that carbohydrate utilization of mammalian kidney is accelerated by phosphate.

Working with winter frogs, Detering (1926) found that surviving kidneys weighing 30 to 40 mgm. (dry weight) removed 1 to 2 mgm. of glucose per hour from the surrounding Ringer solution which contained glucose in 0.01 per cent concentration. On his assumption that over 60 per cent of this glucose was oxidized and if conditions in our experiments otherwise were comparable we should have expected a clear cut increase in respiration as a result of adding glucose. This was actually observed in a few preliminary experiments with bicarbonate Ringer but subsequent experiments, particu-

larly with phosphate Ringer, failed to bear this out (see table 1). The slight increase in average Q_{O_2} produced by glucose addition to phosphate Ringer is hardly significant. It was at first thought that the phosphate might have produced an abnormally high rate of respiration such that glucose addition could cause no further increase. However, Irving (1928) found that glucose utilization in the presence of phosphate Ringer was greater than in bicarbonate Ringer. Moreover in another series of experiments we found that glucose in plain Ringer not only failed to produce an increase but actually caused a significant decrease in respiration.

TABLE 1

The effect of glucose on the Q_{O_2} of kidney in phosphate Ringer*

TEMPERATURE	CONCENTRATION OF GLUCOSE AFTER TIPPING FROM SIDE ARM	Q_{O_2}		
		Before tipping glucose	After tipping glucose	Difference
°C.	*per cent*			*per cent*
20.0	0.08	3.64	3.54	−3.3
24.9	0.05	3.57	4.52	+26.5
21.9	0.05	2.06	2.43	+17.9
21.9	0.05	1.24	1.40	+12.9
23.1	0.05	2.41	2.62	+8.7
23.1	0.05	2.37	2.61	+10.0
20.3	0.05	1.87	1.92	+2.6
21.0	0.05	1.93	2.12	+9.8
21.0	0.05	1.95	1.47	−24.6
21.4	0.05	1.71	2.11	+24.4
21.4	0.05	2.62	2.31	−11.7
Average.....		2.31	2.46	+6.5

* Q_{O_2} equals oxygen consumption in cubic millimeters per milligram dry weight per hour.

TABLE 2

The effect of phlorhizin on the Q_{O_2} of kidney in phosphate Ringer with and without glucose

TEMPERATURE	CONCENTRATION OF PHLORHIZIN AFTER TIPPING FROM SIDE ARM	CONCENTRATION OF GLUCOSE	Q_{O_2}		
			Before tipping phlorhizin	After tipping phlorhizin	Difference
°C.	*per cent*	*per cent*			*per cent*
21.9	0.08	0.13	1.99	1.84	−7.5
21.9	0.08	0.13	3.05	2.49	−18.3
20.5	0.10	0.13	1.65	1.40	−9.9
20.5	0.10	0.13	2.64	2.00	−24.2
22.6	0.10		2.39	2.12	−11.3
20.2	0.10		1.92	1.39	−27.6
20.2	0.10		2.71	2.12	−21.6
21.4	0.025		1.89	2.00	+5.8
21.4	0.025		2.64	2.26	−14.4
21.5	0.05		2.48	2.44	−2.0
21.5	0.05		2.59	2.34	−9.6

Phlorhizin in a concentration of 0.1 per cent produces a 15 to 20 per cent inhibition of kidney respiration whether the tissue is suspended in plain or in phosphate Ringer and whether or not glucose is present (see table 2). Lower concentrations of phlorhizin produce less effect. With concentrations of phlorhizin two to five times stronger than these, Lundsgaard (1933) obtained strong inhibition of esterification of phosphate and of glycogenolysis in muscle, and the inhibitions noted in our experiments might be accounted for on the basis of his explanation of the action of phlorhizin on kidney.

It has been claimed that diuresis produced by substances which are presumably secreted by the tubule cells occasions a marked increase in the oxygen consumption of kidney as a result of the secretory activity (Barcroft and Brodie, 1905; Barcroft and Straub, 1910; Bainbridge and Evans, 1914) but these findings could not be confirmed by more recent investigators (Tamura and Miwa, 1920; Hayman and Schmidt, 1927; Fee, 1929; Gremels, 1929). On the assumption that the addition of substances to excised kidney tissue in respirometers may produce effects on metabolism similar to those produced by these substances *in vivo*, and in view of the fairly general agreement that certain dyestuffs such as neutral red and phenol red are eliminated in the case of the amphibian kidney by a process of active secretion of the tubule cells (see Marshall, 1931), we have studied the action of these dyes on the oxygen consumption of frog kidney. As may be seen from tables 3 and 4, the results were entirely negative. Al-

TABLE 3

The effect of neutral red on the Q_{O_2} of kidney in Ringer solution

Concentration of neutral red = 0.01 per cent

TEMPERATURE	METHOD	Q_{O_2}		
		Control	Experimental	Difference
°C.				*per cent*
18.4	"Kästchen" bicarbonate	1.31	1.33	+1.5
19.7	"Kästchen" bicarbonate	1.78	1.82	+2.8
19.8	"Kästchen" bicarbonate	2.22	1.92	−13.5
19.7	"Kästchen" bicarbonate	1.91	2.13	+11.5
20.6	"Kästchen" bicarbonate	2.38	2.22	−6.9
19.7	"Kästchen" bicarbonate	2.08	2.35	+13.0
19.0	"Kästchen" bicarbonate	2.85	2.96	+3.9
18.1	"Kästchen" bicarbonate	2.38	1.91	−19.5
17.9	"Kästchen" bicarbonate	2.99	3.05	+2.0
Average		2.21	2.19	−1.0
19.1	Volumeter	2.31	1.65	−28.5
19.1	Volumeter	1.49	1.54	+3.4
19.1	Volumeter	1.73	1.82	+5.2
19.5	Volumeter	1.25	1.60	+21.8
19.2	Volumeter	1.43	1.74	+22.0
19.2	Volumeter	1.77	1.82	+3.1
19.5	Volumeter	2.04	1.96	−4.0
19.5	Volumeter	1.88	2.32	+23.4
19.5	Volumeter	2.01	2.31	+15.0
Average		1.77	1.86	+5.7

though the tissues became well stained neither neutral red nor phenol red produced a significant increase in respiration whether the tissues were suspended in plain Ringer, bicarbonate Ringer or phosphate Ringer. These negative results cannot be used however as evidence against the view that these dyes are eliminated primarily by a process of active secretion for it has been shown by Borsook and Weingarden (1931) and by Dock (1933) that in mammals the kidney is capable of increasing its osmotic work enormously without greatly increasing its oxygen consumption. Whether their conclusions, however, which were based on urea secretion, apply also to the elimination of foreign substances such as dyestuffs by the frog kidney is uncertain.

TABLE 4

The effect of phenol red on the Q_{O_2} of kidney phosphate Ringer

TEMPERATURE	CONCENTRATION OF PHENOL RED IN SIDE ARM	Q_{O_2}		
		Before tipping phenol red	After tipping phenol red	Difference
°C.	*per cent*			*per cent*
20.6	0.025	2.07	2.32	+12.5
20.6	0.025	3.10	2.97	−4.2
20.5	0.10	2.42	2.13	−11.5
20.5	0.10	3.00	2.59	−14.0
20.5	0.025	1.82	1.78	−2.0
20.5	0.025	2.84	2.49	−12.0
18.7	0.01	1.71	1.59	−7.0
Average.....		2.42	2.27	−6.2

TABLE 5

The respiratory quotient of frog kidney T = ca. 20°C.

RINGER SOLUTION	RINGER SOLUTION PLUS 0.05 PER CENT GLUCOSE	RINGER SOLUTION PLUS 0.01 PER CENT PHLORHIZIN	RINGER SOLUTION PLUS 0.01 PER CENT PHENOL RED
R.Q.	*R.Q.*	*R.Q.*	*R.Q.*
0.916	0.826	0.625	0.873
0.930	0.823	0.684	0.838
0.800	0.905	0.627	0.942
0.840	0.928	0.677	0.896
0.833	0.930	0.780	
0.920			
0.895			
Av..0.876	0.882	0.681	0.887

2. Respiratory quotient. Barcroft and Brodie (1905) found the R.Q. of mammalian kidney *in situ* to be unity. Richardson, Loebel and Schorr (1926) using manometric methods obtained the value of 0.823. They also found that glucose produced a definite rise in the R.Q., the average figure being 0.881. For frog kidney we obtain an average R.Q. of 0.88 and glucose addition fails to raise this figure appreciably. It must be remembered, however, that the experiments were done for the most part on summer frogs. It is possible that similar experiments on winter or starved frogs might show an increase.

Addition of phlorhizin to kidney tissue produces a marked drop in the value of the R.Q., the average being 0.68. A decrease in R.Q. of the entire body with phlorhizin has long been known but was naturally attributed to depletion of carbohydrate. This striking decrease in the R.Q. of excised kidney with phlorhizin in a concentration of only 0.01 per cent was hardly

to be expected, for, according to Lundsgaard, the phlorhizin interference with carbohydrate metabolism through inhibition of phosphate esterification takes place only in concentrations twenty to fifty times that here employed. Phenol red in a concentration of 0.01 per cent, which is sufficient to cause coloration of the tissue produces no change in the R.Q., the average being 0.87.

SUMMARY

1. The Q_{O_2} of kidney from summer frogs was found to be about 1.80 in plain Ringer, 2.20 in bicarbonate Ringer, and 2.50 in phosphate Ringer. Addition of glucose caused only very slight increase in the Q_{O_2} while phlorhizin produced a decrease of 15 to 20 per cent. Neutral red and phenol red caused no significant increase.

2. The R.Q. of frog kidney in plain Ringer was found to be 0.88. Glucose addition did not change the figure significantly but phlorhizin decreased it to 0.68. Phenol red had no effect on the R.Q., the average being 0.87.

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THE RESULTS OF FEEDING LECITHIN AND PANCREAS IN DEPANCREATIZED DOGS ON THE LIVER FAT AND ITS SAPONIFIABLE AND UNSAPONIFIABLE FRACTIONS

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In 1931 Hershey and Soskin (1) and later Best and Hershey (2) reported that lecithin fed to depancreatized dogs prevented or cured the condition of "liver failure," which they had observed in such animals. The effect of lecithin and its components (3) on fat deposition in the livers of normal rats was studied and it was found that choline and also betaine inhibited the deposition of liver fat in rats.

During the past three years in this laboratory we have observed depancreatized dogs on a basic diet of lean beef (150 gm.), cracker meal (100–125 gm.), sugar (20–30 gm.), skimmed milk powder (0–30 gm.), dried brewer's yeast (5–10 gm.), bone ash (4 gm.), and the Cowgill (4) salt mixture (3 gm.). To this diet has been added cod liver oil alone, cod liver oil and lecithin alone, carotene in maize oil, and raw pancreas (75–100 gm.). Mead Johnson cod liver oil and carotene were used. The lecithin was crude egg yolk lecithin obtained from John Carle and Son. On analysis in our laboratories we found it to contain total fat 60.031 grams per cent, of which 4.682 grams formed the unsaponifiable fraction.

At the end of the period of observation the animals were killed by exsanguination and the liver removed immediately. The liver lipids were determined by the Mayer and Schaeffer modification of the Kumagawa and Suto method, the unsaponifiable fraction by the Liebermann method (5). All determinations were done in duplicate on 10 gram samples of liver, which were weighed immediately. The results of our analysis are the average of the two samples, and no results were accepted that did not check in the first decimal place. Twenty depancreatized animals are reported. All of these received the basic diet. In four animals cod liver oil and lecithin were added to the diet; in five lecithin alone was added; in three cod liver oil and pancreas; in three carotene in oil and pancreas; in three the diet was given with pancreas as the only addition and two dogs received 200 grams of raw beef liver in place of lean beef 150 grams. These two dogs also received pancreas. One of the dogs, no. 10, received

the cod liver oil concentrate in place of the whole oil. All of the animals received insulin during the entire diabetic period, but not enough to keep them sugar free, table 2.

TABLE 1

Total fatty acid, saponifiable and unsaponifiable fractions of normal dog livers

DOG NUMBER	TOTAL FAT	SAPONIFIABLE	UNSAPONIFIABLE	BASIC DIET AMOUNTS IN GRAMS	ADDITIONS TO DIET	TOTAL TIME OBSERVED
	grams per cent	*grams per cent*	*grams per cent*			*days*
B	3.350	2.388	0.962	Kitchen scraps	None	240
C	2.535	1.838	0.697	Kitchen scraps	None	240
D	1.905	1.773	0.132	Meat 150 Cracker meal 150 Whole milk 100 Lard 15 Salt mixture 3	Cod liver oil 4 cc. daily for first 10 days. Total 40 cc.	33
E	2.048	1.767	0.281	Meat 150 Cracker meal 100 Sugar 30 Yeast 5 Salt mixture 3 Bone ash 4	Cod liver oil 10 cc. daily for first two weeks. Total given 140 cc. Lecithin 10 grams daily for 81 days. Total given 810 grams	107
F	3.290	2.951	0.340	Meat 150 Cracker meal 100 Sugar 30 Skimmed milk powder 30 Yeast 5 Bone ash 4 Salt mixture 3	None	134
G	3.376	2.851	0.525	Liver 200 Cracker meal 100 Skimmed milk powder 30 Sugar 30 Yeast 5 Bone ash 4 Salt mixture 3	Liver in place of lean beef	74

In addition to the depancreatized dogs, six normal dogs are reported, in table 1. Two of these animals were fed a mixed diet of kitchen scraps, the absolute values of which are not known. Of the other four dogs on the basic diet, one received cod liver oil for a short period of time, one cod liver oil and lecithin, one beef liver in place of lean beef and one the diet alone.

TABLE 2

Total fat, saponifiable and unsaponifiable fractions of livers of depancreatized dogs

	DOG NUMBER	TOTAL FAT	SAPONIFIABLE	UNSAPONIFIABLE	TOTAL C.L.O.	INTAKE LECITHIN	NUMBER OF DAYS FED	DIABETIC PERIOD	AVERAGE 24-HOUR URINE SUGAR		AVERAGE DAILY INSULIN DOSE	
									Before lecithin	During lecithin	Before lecithin	During lecithin
		grams per cent	*grams per cent*	*grams per cent*	*cc.*	*grams*	*days*	*days*	*grams per cent*	*grams per cent*	*units*	*units*
Cod liver oil and lecithin	1	4.150	3.771	0.379	2180	1090	109	142	16	27	8	28
	2	4.116	3.506	0.610	1120	560	56	56		14		10
	3	16.412	15.825	0.587	600	200	20	102	15	8.5	18	19
	4	8.060	7.685	0.375	1540	770	77	77		19		28
Lecithin	5	11.051	9.990	1.061	0	120	12	61	26	22	11	8
	6	3.648	2.789	0.859	0	1150	115	115		15		10
	7	2.162	1.402	0.760	0	70	7	35	15	9	7	6
	8	2.048	1.767	0.281	0	140	14	19		15		7
	9	2.349	1.833	0.516	0	930	93	93		14		17
									During period of diabetes		During period of diabetes	
					(tab.)							
Cod liver oil and pancreas	10	3.810	3.043	0.767	246	0	41	62	6		4	
	11	3.779	3.428	0.351	720	0	24	80	21		32	
	12	4.997	4.591	0.406	1760	0	88	88	30		25	
					CAROTENE IN OIL							
					cc.							
Carotene and pancreas	13	3.378	2.792	0.586	50		72	123	49		23	
	14	3.777	3.257	0.520	34		34	75	28		10	
	15	3.291	2.690	0.601	44		44	73	17		8	
Pancreas	16	3.367	2.743	0.624				154	37		12	
	17	2.980	2.148	0.832				62	15		8	
	18	4.576	3.775	0.801				10	41		12.5	
Liver and pancreas	19	6.329	5.828	0.501				41	18		10	
	20	4.512	3.999	0.512				81	27		31	

RESULTS AND DISCUSSION. The results on the normal dogs are reported in table 1 and on the depancreatized dogs in table 2. In the depancreatized dogs, when cod liver oil, lecithin or carotene were not administered for the entire diabetic period their administration covered the latter part of this period. The length of time of their administration is indicated in the table. During the period prior to lecithin administration in dogs 3, 5 and 7, raw pancreas (75 gm.) was fed daily. The animals are grouped according to the additions made to the basic diet. Also reported in table 2 are the average 24-hour excretion of sugar in grams and the average daily dose of insulin. When lecithin was given for only part of the period of observation the excretion of sugar and the units of insulin are calculated for both before and after the administration of lecithin.

In the group of normal dogs the per cent of total liver fat varied from 1.905 grams per cent to 3.376 per cent. Of these six dogs only E received lecithin. The total liver fat in this dog was 1.285 grams lower than the average of the total fat in dogs F and G. We compare dog E to these two dogs because the diet in dogs B and C was not measured and dog D was only observed for 33 days. This dog incidentally had the lowest total fat of the group, which might be due to the short time she was on the diet. With this exception the dogs on lecithin did have the lowest total liver fat and the lowest unsaponifiable fraction, but the difference is certainly not striking.

In considering the depancreatized dogs we wish to point out that we have not encountered the symptoms of "liver failure" described by the Toronto observers. In all probability this is due to the fact that all of our dogs received raw pancreas daily. The urine was examined daily for bile and the test was not positive. All of our animals were autopsied and a careful examination made for any remaining pancreatic tissue. In none of this group of dogs was any found. The question might be raised that our animals were not observed for sufficiently long periods. However, Hershey and Soskin report that the disturbance of liver function may appear as early as six weeks after pancreatectomy and in the protocols of their animals report the urine positive for bile 19, 22 and 29 days post-operative. Apparently time is not necessarily a factor and apparently raw pancreas prevents the appearance of these symptoms. As a group the depancreatized dogs had a higher total liver fat than did the normals. However, in the entire group of diabetic animals the per cent of total fat was markedly increased in only four dogs, 3, 4, 5 and 19. As compared to the normal dogs where the total fat did not exceed 3.376 grams per cent, five more diabetic dogs had a slightly elevated total fat, the highest being 4.997 grams per cent. Of the four dogs with a very high total fat, three received lecithin and one was on raw beef liver in place of lean beef. Of the three receiving lecithin two, nos. 3 and 4, received cod liver oil, and

one, no. 5, received lecithin alone. If we compare the dogs on pancreas and cod liver oil, it is obvious that lecithin was less effective in preventing the deposition of liver fat than was pancreas. For example, nos. 3 and 4, on lecithin, received 600 and 1540 cc. of cod liver oil, while dogs 11 and 12 on pancreas received 720 and 1760 cc. of the oil, and yet the total liver fat was lower. The other two dogs on lecithin and cod liver oil had low total fats but the average, 4.133 grams per cent, was only 0.255 gram lower than the average of 11 and 12. Comparing the per cent of liver total fat in the dogs on the diet plus lecithin with the dogs on the diet plus pancreas, the group on lecithin, with one exception, 5, had a lower total fat, the average being 2.55 grams as compared to 3.64 grams in the dogs on pancreas. From these results it seems that lecithin is less effective than raw pancreas in preventing the deposition of liver fat when fat is given, in the form of cod liver oil, but that on a low fat diet lecithin may maintain the liver fat at a slightly lower level than raw pancreas. The dogs with the very high per cent of total fat might be considered as having suffered from "liver failure" although objectively they displayed no symptoms. The dogs receiving carotene in oil and pancreas had no increase in liver fat over the dogs on pancreas alone. Of the two dogs on raw liver and pancreas one had the fourth highest liver fat of the entire group and the other was within the higher limits. Liver, of course has a higher phospholipid content than lean beef.

As regards the unsaponifiable fraction of the total fats in the depancreatized dogs, the average of the dogs on cod liver oil and lecithin was 0.488 gram; on lecithin alone 0.695 gram; on pancreas and cod liver oil, 0.379 gram, but when the cod liver oil was given as the concentrate the unsaponifiable fraction was 0.767 gram; on pancreas 0.615 gram and on liver and pancreas 0.506. In view of the fact that the dogs on lecithin alone had a somewhat lower total fat, it is interesting that such a large per cent of it was unsaponifiable.

The next point to consider is the effect of lecithin on the excretion of sugar. Of the nine dogs receiving lecithin, five received it during their entire diabetic period, nos. 2, 4, 6, 8 and 9. These dogs may then be compared to dogs receiving raw pancreas during their entire diabetic period. The dogs on lecithin excreted an average of 15.4 grams of sugar daily and received 14.4 units of insulin daily. Omitting the two dogs on raw liver, as it contains some lecithin, the dogs on pancreas spilled 27 grams of sugar daily and received 15.1 units of insulin. In the four dogs who received lecithin during only part of their diabetic period, the average excretion of sugar before the administration of lecithin was 18 grams and the insulin 11 units. After lecithin the sugar excretion was 16.6 grams daily and the insulin 15 units. Judging from these results the animals on lecithin for their entire diabetic period were somewhat less severely diabetic

than the animals on pancreas. In the animals receiving lecithin for only part of their diabetic period there was no very striking effect on the glycosuria. Examined individually, two of the dogs in this group, nos. 3 and 7, excreted less sugar following lecithin; in one the glycosuria increased and the fourth showed little change. In this respect our results do not confirm those of Hershey and Soskin, who found sugar excretion increased on the addition of lecithin. This they attribute to a regaining, on the part of the liver, of its power of forming sugar presumably through an increased production of sugar from protein or fat. However, there is as yet no evidence that the phospholipid lecithin can stimulate the production of glucose from either protein or fat and our results do not substantiate any such hypothesis. Were this the case the dogs on lecithin should have required more insulin to control their glycosuria.

The question of liver dysfunction in the diabetic animal must, we think, be considered from a different angle, now that the liver is no longer looked upon as the seat of the desaturation of the fatty acids (6 and 7). We are not at variance with the suggestion that there may be some liver dysfunction in such animals, but as yet no proof has been advanced as to the nature of such a dysfunction. Recent work has shown that the pancreatic lipase has no great effect on the hydrolysis of fat in the intestine (8 and 9), so that its loss in the depancreatized dog need not be considered as an important factor. Drummond in 1933 (7) pointed out that there is increasing belief that particulate absorption occurs, and that fatty substances can gain direct access to the portal circulation, and we may infer that this is not interfered with in these animals. In the group of animals reported here, additional proof that the fat given as cod liver oil was absorbed is that the vitamin A content of their livers was very high as compared with the vitamin A content of the livers of the animals not receiving any source of the vitamin (Ralli et al., 1934, 10). The one exception to this was dog 3, who had a low vitamin A content and a high liver fat. One might, we think, consider the disturbance of liver function a relative one due perhaps to a flooding of the tissues by fat with the liver in consequence flooded the most and absorbing the most. Over a long period of time or where the flooding was excessive due to ingestion or to an inability to utilize fat, the per cent of liver fat must necessarily increase. Whether this reaches a state where pathological dysfunction occurs is a point that needs elucidation. Our results, in any case, indicate that whatever dysfunction of the liver, as evidenced by increasing fat deposition, does occur is as well controlled by raw pancreas as by lecithin.

SUMMARY

The results of fat analyses of the livers of twenty depancreatized dogs are reported. Nine of these dogs received crude egg yolk lecithin and

eleven raw pancreas, in addition to the basic diet. In the dogs who received cod liver oil and lecithin, as compared to the dogs receiving cod liver oil and raw pancreas, lecithin was not as effective as pancreas in preventing the deposition of liver fat. The dogs on lecithin alone had a slightly lower per cent of liver fat than the dogs on pancreas.

The results of the fat analysis of six normal dogs are also reported. The total liver fat was lower in this group than in the depancreatized. Lecithin, fed to one dog, had no very striking effect on the per cent of total fat.

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ON THE VARIABILITY OF THE H/T RATIO IN THE TWITCH OF ISOLATED SKELETAL MUSCLE AND THE PROOF OF THE VALIDITY OF THE METHOD USED FOR MEASURING HEAT PRODUCTION

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In a recent paper on the heat production of cardiac muscle (1) it was pointed out that, while it is evident that there is a mean constant value for the ratio of heat production to tension developed, around which the individual values fluctuate, yet from time to time under the same constant conditions deviations occur which are too large to be ascribed to experimental error. This was found to be true for both cardiac and smooth muscle, both under constant conditions of oxygen-want and oxygen-plenty. It was concluded therefore that the mechanism of the contractile act, whatever its nature may be, at least for these two kinds of muscle could not be regarded as one of any great precision. But rather are the energy input and energy output relations more like those between a stream and the mill-race that it feeds. Is this also the case with skeletal muscle?

Investigations on this problem during the last two decades, in their summaries at least, teach that the H/T ratio, under constant, favorable conditions is a constant, implying thereby that any observed individual variations from this constant are within the experimental error. In the present paper the author wishes to submit evidence that skeletal, like cardiac and smooth muscle, exhibits variations in its heat/tension ratio which also are too large to be ascribed to experimental error.

The muscles studied were taken from Rana pipiens and the methods employed the same as were used in the work on heart muscle (1) excepting that, besides the Moll, the Zernicke (Z_c-type) galvanometer was used. The parts of the method to be emphasized are the use of a constant temperature bath (the temperatures in the various experiments varied between 8 and 15°C., and in no one experiment more than a half degree in four hours;) of iron-constantan 60-junction thermopiles carefully constructed following the Bürker-Hill experiences and as modified by Gemmill and Snyder (2); and especially the use of the method of direct measurement of galvanometer-deflection-time areas for the determination of heat-production quantities, and finally the reliance on the theoretical characteristics of galvanome-

ter and thermopile (frequently calibrated) for the conversion of these quantities into absolute heat units. The method of measuring and evaluating the areas was reported and partly described in the above mentioned paper on heart muscle (1) and the fundamental proofs of its validity were reported at the last Annual Meeting of the American Physiological Society.[1] But since the abstract (3) of this latter report did not include all the evidence submitted at that time it is essential that it be herewith incorporated.

The method is based on the proposition that the total area of the galvanometer deflection-time curve is a measure of the total heat liberation, and that such a curve can be more easily measured directly by a planimeter than by the method heretofore used, namely, that of harmonic analysis.

If all the conditions requisite for the recording of temperature changes of isolated muscle by means of the differential thermopile and galvanometer are observed, then usually when the muscle is in oxygen-want, and sometimes when it is in oxygen-plenty, it is observed that the heat is all liberated and the temperature rise of the muscle reaches its maximum in the first few seconds following the beginning of tension rise. In this case the graphic records of the cooling of the muscle present curves of the simple "die-away" form, such as one would expect from the cooling of a warmer body, to which heat is no longer added, and when surrounded by a cooler medium. Let us call this the normal cooling curve. Such curves are illustrated by the thermomyograms reproduced here in figure 1 in the series marked A, C and D. As a rule in oxygen-plenty, and sometimes in oxygen-want, however, the thermomyograms are no longer of the normal cooling form. Heat being liberated in the muscle irregularly for several minutes after the mechanical change is finished, the curve is prevented from taking the usual course to the base line, as is seen by the thermomyograms in series B of figure 1. If in any one experiment a number of thermomyograms of the normal cooling type are obtained and their areas measured, it is found that these areas bear a fairly constant relation to the corresponding amounts of heat which produced them, then it is obvious that unit area could be given a definite value in terms of heat. The constant thus derived could then be used to calculate the heat represented by thermomyograms of the same

[1] Only now after this paper has been sent to press have I become aware that I have been anticipated in this method. Prof. A. V. Hill in 1928 had already stated the proposition and given its theoretical basis. He also then briefly described a method for calibrating the heat equivalent of unit area of the galvanometer deflection-time curves and used it for total heat determinations in muscle in several investigations (Proc. Roy. Soc. B, **103:** 117–191). On page 121 of his report it is stated that the theoretical relations involved have been verified experimentally, but none of the experimental proof is submitted. This I have done independently and no doubt quite differently in the present paper. It further turns out that my method for "area calibration" of thermomyograms is distinctly different from that of Professor Hill's.

experiment that contain delayed heat. As will be seen in what follows this expectation is fulfilled both in the controls and in contracting muscle.

The warm junctions of a thermopile were covered with paraffin in which a coil of manganin wire had been imbedded. Known amounts of electricity

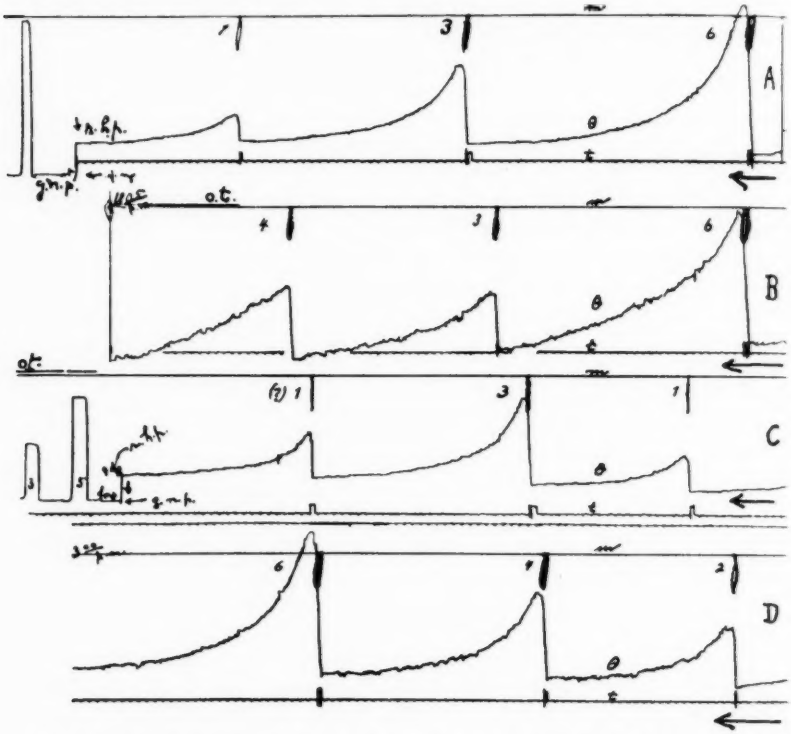

Fig. 1. Thermomyograms from the experiment of 1/25–26/34 in mirror images reduced about 44 per cent from the size of the originals. Read from right to left. The trace lines marked θ are the thermomyographic responses; those marked m are the corresponding mechanograms, the down strokes are the twitches; those marked t are time signals marking 20-seconds intervals, and indicating moment of electrical stimulation of the nerves. The numerals near the down strokes of m indicate the number of single shocks sent into the nerves of the muscles, a pair of gastrocnemius. For further explanation see text.

were sent through the coil and graphic records taken of the temperature changes in the paraffin as registered by a Moll galvanometer. Upon analyzing the records and plotting results we get graphs showing that the areas of the thermo-electrograms tend to be linear to the total heat liberated by the heating coil. In figure 2A it will be noted that the relation holds

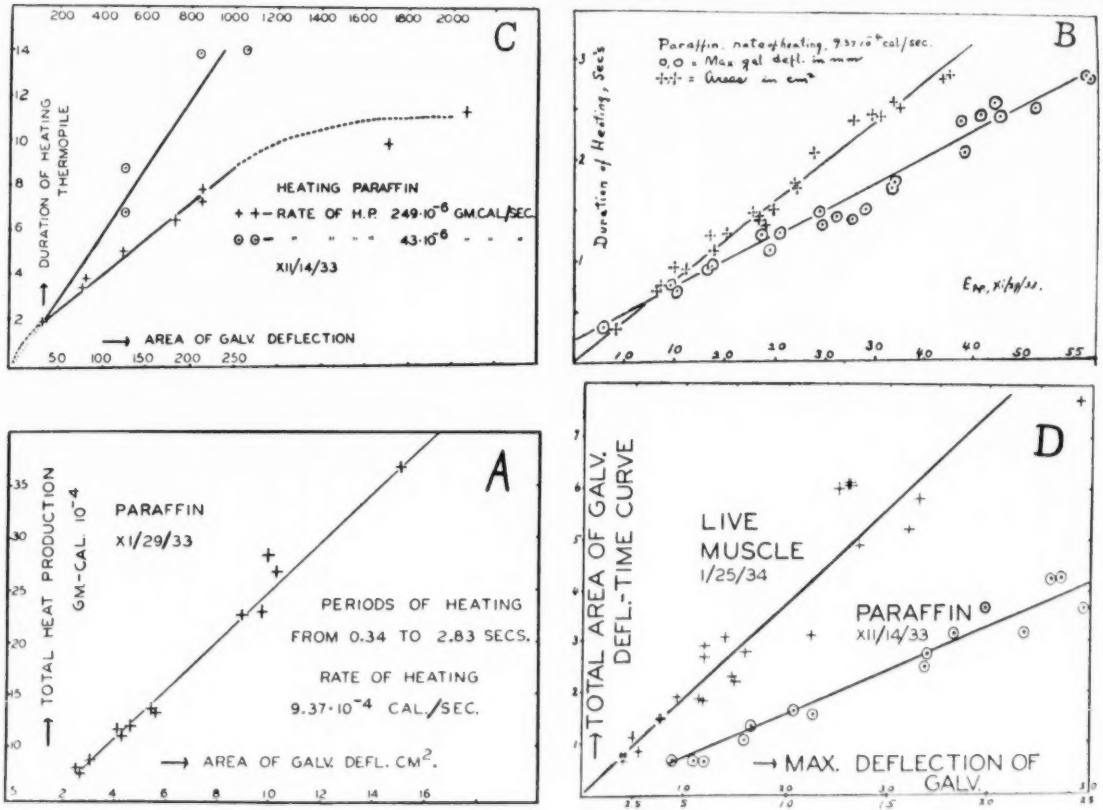
C
DURATION OF HEATING THERMOPILE
HEATING PARAFFIN
+ + — RATE OF H.P. 249·10⁻⁶ GM.CAL/SEC.
⊙ ⊙ — " " " " 43·10⁻⁶ " " "
XII/14/33
AREA OF GALV. DEFLECTION
B
Paraffin. rate of heating 9.37/10⁻⁴ cal/sec.
0.0 = Max gal defl. in mm
+·+ = Areas in cm²
Duration of Heating, Secs
A
PARAFFIN
XI/29/33
TOTAL HEAT PRODUCTION GM-CAL. 10⁻⁴
PERIODS OF HEATING FROM 0.34 TO 2.83 SECS.
RATE OF HEATING 9.37·10⁻⁴ CAL./SEC.
AREA OF GALV. DEFL. CM².
D
LIVE MUSCLE 1/25/34
PARAFFIN ⊙ XII/14/33
TOTAL AREA OF GALV. DEFL.-TIME CURVE
MAX. DEFLECTION OF GALV.

Fig. 2. See text for explanation.

for durations of heating varying from 0.3 second to nearly 2.8 seconds when the rate of heating is about 0.001 calorie per second, and that both maximum galvanometer excursion and area remain linear with increased duration of heating as shown in figure 2B. If the heating rate is reduced to $4.3 \cdot 10^{-5}$ cal./sec., the duration of heating may be extended to nearly 14 seconds and the relation still remains linear, as will be seen in the curve to the left in figure 2C. The limits of this linear relation are brought out by raising the rate of heating, say to the rate of $25 \cdot 10^{-5}$ cal./sec., as appears in the curve to the right of figure 2C. Detailed data of one of these experiments are assembled in table 1. Here are shown the ratios of 1, the maximum temperature rise, as determined from the maximum galvanometer deflection, to the total heat liberated in the paraffin; 2, the total heat to the total area of the galvanometer deflection-time curve. Both these ratios tend to a constant. The value of the T/H ratio approximates the reciprocal of the specific heat and the weight of the paraffin as would be expected after all the physical factors involved are considered. The deviation of the individual H/A ratios are also small enough to permit taking their mean as a constant. This is the constant which I believe, when determined anew for each experiment, can be used in calculating the total heat of any other set of thermo-electrograms taken under the same conditions.

The experiments heating paraffin warranted the extension of the method to the analysis of the graphic records obtained from contracting muscle. Figure 2D, in the curve to the left, gives the results obtained from a pair of gastrocnemius muscles of frog made to contract in oxygen-want. Here also one observes that area tends to be linear with extent of galvonometer response, and that the scatter of the individual readings is not much greater than in the case of heating paraffin.

This linear relation between vertical extent and total area also holds within limits with varying amounts of tension developed by the muscle; this includes summing the tensions of several twitches in succession if they are not too widely spaced in time and not more than 4 or 5 in a series for a single thermomyogram, as will be seen in the thermomyograms reproduced in figure 1 and in the plotted results in figure 3. In this latter figure are plotted the data from an experiment with a pair of gastrocnemius muscles with their nerves attached and in complete oxygen-want. Maximal induction shocks applied to the nerves were used to evoke single twitches or groups of twitches spaced just far enough to prevent summation. The data in the figure are given in arbitrary units. The results from similar experiments with pairs of rectus abdominalis and sartorius muscle of frog stimulated directly present the same linear relation in the thermomyograms of the normal cooling type.

The detail in the determination of the heat value of unit area of thermomyograms is illustrated in table 2. This table contains the data from a

pair of gastrocnemius muscle stimulated through their nerves for all the thermomyograms of the normal cooling type. It will be noted that some of these were taken while the muscle was in oxygen-plenty. The readings have been converted into absolute energy units. Under the rubric gd/gA is given the ratios of maximum galvanometer deflection to total area of thermomyogram. The ratios tend toward a constant with mean for the 14 readings of 5.77, a figure that may be converted into heat units at once

TABLE 1*

Heating paraffin

MAX. RISE IN TEMP., DEG. C. $\times 10^{-4}$ (T)	TOTAL HEAT LIBERATED IN THE PARAFFIN, GM.-CAL. $\times 10^{-4}$ (H)	$\frac{T}{H}$ DEG. TEMP. RISE PER UNIT OF HEAT	$\frac{H}{A}$ HEAT PER MM. GM.-CAL. $\times 10^{-4}$
14.35	2.90	4.96	2.28
26.48	6.05	4.38	2.30
18.16	3.76	4.83	2.98
25.52	5.96	4.28	2.84
53.93	12.70	4.24	2.55
75.27	17.92	4.20	2.12
36.05	8.22	4.39	2.65
75.30	19.20	3.94	2.26
63.19	15.93	3.97	2.16
54.32	11.80	4.60	2.07
68.55	14.96	4.58	2.35
58.40	14.56	4.01	2.31
79.10	18.50	4.27	2.54
		(13) 4.357	(13) 2.476

* At the end of the experiment the paraffin covering the warm junctions of the thermopile was scraped off and weighed. The weight found was 0.319 gram. The specific heat of paraffin is given as 0.6939. By theory the mean value found for the ratio, T/H, ought to be the product of the reciprocals of the weight and the specific heat of the substance heated. From this the weight of the paraffin was probably more nearly the product of the reciprocals of T/H (as found above) and the specific heat, or 0.331 gram; which indicates that all the paraffin was probably not recovered by the method used. It is worth noting that the extreme deviations of the ratios T/H and H/A in the table vary respectively ± 11.5 per cent and ± 17.5 per cent from their mean values.

by multiplying by $1.276 \cdot 10^{-4}$, the constant gotten from the characteristics of thermopile and galvanometer, and the specific heat and weight of the muscle. Or, as was done, each of the individual readings is multiplied by this constant, thus obtaining the heat that gave rise to the several galvanometer deflections, and therefore by our definition also the total heat represented by the several areas of the thermomyograms appearing under A. The ratio of Hgd/A then gives the heat value per cm.^{2} of area, the mean

value of which is $7.49 \cdot 10^{-4}$ gm.-cal. The mean value of gd/gA put in heat units is 5.77 x $1.276 \cdot 10^{-4}$, or $7.32 \cdot 10^{-4}$; the mean value of the two extremes of the series is $7.51 \cdot 10^{-4}$ gm./cal. The extreme deviations of the Hgd/A values are ± 17.8 per cent from their mean. Referring back to the paraffin experiment, table 1, we find that the Hgd/A extreme deviations there also vary about ± 17.85 per cent. The experimental error therefore when muscle

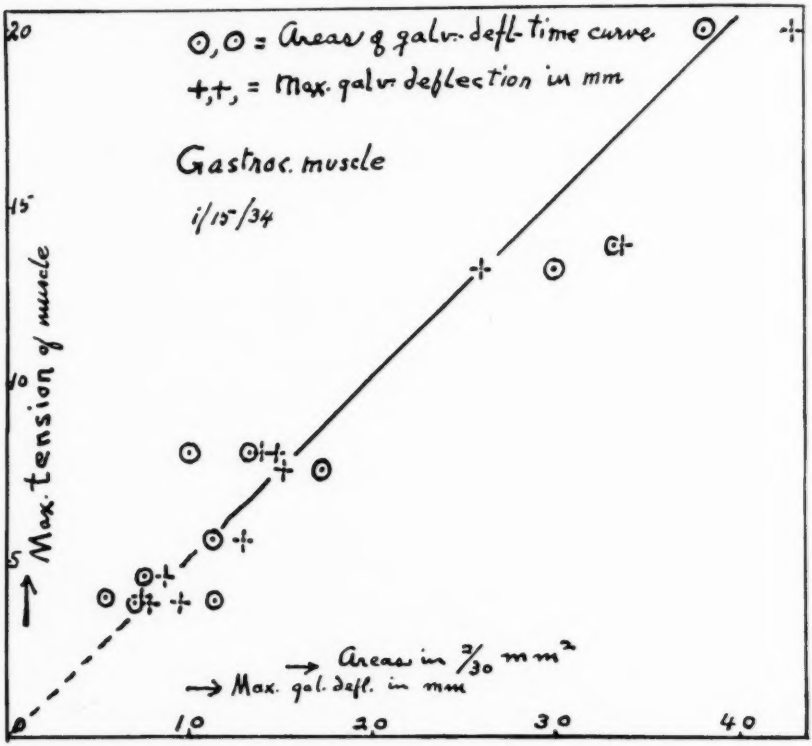

Fig. 3. See text for explanation.

is warmed by its own heat production is probably no greater in any case than say ± 20 per cent.

As a corollary it is of interest to note that just as in the case of paraffin so with muscle, the ratio of maximum temperature rise (as determined by maximum galvanometer deflection) to total heat production (as calculated from total thermomyogram area, when delayed heat is absent) gives a figure that approaches the reciprocal of the product of the specific heat into the weight of the substance heated, as will be seen in table 3. This relation

TABLE 2

Experiment of February 19–20, 1934. Analysis of thermomyograms showing a minimum of delayed heat production

GASEOUS MEDIUM	TIME IN HR., MIN.	MAXIMUM TENSION (T_m)	gd mm.	gA cm.²	$\frac{gd}{gA}$	$\frac{Hgd}{gA}$	Hgd*	HgA†	HgA − Hgd‡	$\frac{Hgd}{T_m}$
						cal. × 10^{-4}				cal. × 10^{-4}
		gm.								
Oxygen-	2/19									
plenty:	11:22	749	25.6	4.47	5.68	7.32	32.7	32.8	0.1	4.37
		381	15.8	2.50	6.32	8.16	20.4	18.3	−1.9	5.30
		764	26.0	4.00	6.50	8.30	33.2	29.3	−3.9	4.35
		1392	39.2	7.28	5.38	6.83	49.7	53.4	3.7	3.54
		783	22.8	3.29	6.93	8.85	29.1	24.1	−5.0	3.74
	12:40	1579	36.8	6.66	5.53	7.04	46.9	48.8	1.9	2.97
Nitrogen:	*12:45*									
	8:40	2074	47.6	9.26	5.16	6.56	60.8	67.9	7.1	2.93
		1494	28.3	2.99	5.17	7.25	36.2	36.6	0.4	2.42
		1399	18.6	3.23	5.26	7.34	23.7	23.7	0.0	1.70
		546	11.9	2.02	5.84	7.53	15.2	14.8	−0.4	2.78
	9:15	1511	28.5	4.53	6.29	8.03	36.4	33.2	−3.2	2.41
Nitrogen:	2/20									
	3:00	2215(?)	29.0	5.30	5.47	6.98	37.0	38.8	1.8	(?1.67)
		1414	26.5	5.47	4.85	6.18	33.8	40.1	6.3	2.39
	3:44	463	8.5	1.28	6.64	8.44	10.8	9.4	−1.4	2.33
					(14)5.77	7.49			0.364	(7)2.42
					Mean of extremes, 5.89	7.51				

Note, gd in the captions of various rubrics is an abbreviation for maximal initial galvanometer deflection; gA, for total area of the galvanometer deflection-time curve; T_m for the maximum total tension developed by the muscle. Total tension here means the initial tension plus the maximum tension that is recorded by the maximum excursion of the muscle lever. In the cases where two or more twitches were evoked to produce a single thermomyogram, the tensions of all the twitches were summed.

* Hgd is the constant representing the combined characteristics of galvanometer and thermopile times the galvanometer excursion times the weight and specific heat of the muscle contracting. In this case Hgd = gd × $2.671 \cdot 10^{-4}$ × 0.825 × 0.580 = gd × $1.276 \cdot 10^{-4}$.

† HgA is the total heat produced and is the product of the mean value of Hgd/gA and the total area of the thermomyograms; that is, HgA = $7.49 \cdot 10^{-4}$ × A.

‡ The differences between Hgd and HgA in this column are measures of individual deviation from the mean value of Hgd rather than a measure of "delayed heat." By definition in this table Hgd and HgA should be equal.

becomes clear when we consider that the heat, as determined from maximum temperature rise of the substance heated and the characteristics of galvanometer and thermopile, is made equal to the heat as determined by the area of the galvanometer deflection-time curve. Their ratios therefore ought to be unity; and if so, then the ratio of maximum temperature rise to total heat (as determined by the two methods) ought to be equal to the product of the reciprocals of specific heat and weight of substance heated. By experiment this is found to be the case for contracting muscle as well as heated paraffin, as the data in table 3 indicate. For further detail of the method the reader is referred to the paper on heart muscle mentioned above (1).

TABLE 3

Mean of ratios of maximal temperature rise to total h.p. in thermomyograms containing minimal delayed heat

DATE OF EXP.	KIND AND WT. OF MUSCLE	NUM. OF OBS'TS (n)	$\frac{\Theta gd}{HgA}$	$\frac{1}{sp.\ h \cdot W_m}$	P.E. OF MEAN, IN ± PERCENT*
1934	*grams*				
1/9	Gastrocn., 0.85	7	1.402	1.425	
1/15	Gastrocn., 0.89	9	1.383	1.365	
1/25-26	Gastrocn., 0.99	11	1.224	1.224	3.07
2/19-20	Gastrocn., 0.58	14	2.160	2.088	1.85
2/23	Sartorius, 0.105	10	1.150	1.154	0.36
1933					
12/14	Paraffin control	13	4.36	4.52	2.15

* In these the mean $= A \frac{\Sigma(\sigma f),}{n}$ and the standard deviation is taken as $\sigma = \pm \sqrt{\frac{\Sigma(x^2 f).}{n}}$ The probable error of the mean (A) is put as P. E. $= \pm \frac{2}{3} \cdot \sqrt{\frac{\sigma}{n-1}}$, and this value finally determined as a percentage of A.

It is concluded therefore that, whenever one wishes to know the total heat production of a whole muscle action of short duration or the part produced during relatively long phases of this whole action (as during the contraction-relaxation phase of muscle kept at the lower ranges of temperature) the method of measuring areas of thermomyograms directly is a valid one and may be substituted for the more tedious and difficult method of harmonic analysis.

Let us then proceed to the manner of the application of the method to thermomyograms that include heat liberated after the tension change is over. Such records have been assembled from the protocols of the same experiment from which the data dealt with in table 2 were gotten. The results after analysis are put in table 4 in which those relating to the muscle

while in oxygen-plenty appear in the lower half, those relating to the muscle while in oxygen-want appear in the upper half of the table. The figures under the rubric, Hgd, give the initial heat again as calculated from maximum galvanometer deflection, those under HgA the total heat as calculated from the total areas of the thermomyograms times the constant, $7.49 \cdot 10^{-4}$, which was found in table 2 to be the heat value per centimeter^2 of area.

As will be noted the differences between the HgA and Hgd values while the muscle was in nitrogen are small notwithstanding the fact that these thermomyograms by inspection gave marked evidence of containing delayed heat. A few of them show considerable difference. On the other hand most of the cases with the muscle in oxygen-plenty show large differences in the HgA—Hgd values and only a few show small differences. Now if we assume that Hgd represents "initial heat" and HgA "total heat" then for the cases in table 4 the difference between the two must be the "delayed heat." In table 2, however, this difference can only mean the chance deviations due to operational or experimental error or both; for by definition the values of Hgd and HgA in this table should be equal and their difference zero. As it turned out they varied from -5.0 to $+7.1$ with a mean value of $+0.364 \cdot 10^{-4}$. It will be noted however that in none of the cases in table 2 does this difference exceed 20 per cent of the corresponding Hgd value itself, which we pointed above was the probable limit of experimental error.

In table 4 we are dealing with thermomyograms that already by inspection gave evidence of including heat of delayed origin and the difference between HgA and Hgd accordingly is often very marked. In all these records the galvanometer had returned to the base-line fully 100 per cent. This difference therefore may well be considered a measure of the delayed heat in each case. That the difference is very small in some of the cases for the muscle in oxygen-plenty and rather large in a few cases for the muscle when in oxygen-want can only be evidence that either delayed heat is not always produced promptly after the twitch, or that it is occasionally absorbed by variable concomitant endothermic reactions, or that the initial heat while usually in greater excess is sometimes cut down to a quantity more nearly approaching the bare energy requirement for the tension developed.

These variations from mean values are also to be found among the ratios of heat production to tension developed, as appears in the last columns of both tables 2 and 4 under the rubrics Hgd/T_m and HgA/T_m. These ratios, especially under anaerobic conditions, tend also to fluctuate about a mean. In table 2, where we would expect to find very little delayed heat, the extreme ratios of Hgd/T_m vary 27 per cent from their mean, whereas the extremes in tension taken alone vary nearly 64 per cent from their mean.

In table 4 the deviation of the heat/tension ratios under oxygen-want is somewhat greater and under oxygen-plenty markedly greater. In the

TABLE 4

Experiment of February 19–20, 1934. Analysis of thermomyograms which include marked delayed or recovery heat production

GASEOUS MEDIUM	TIME	MAXIMUM TENSION Tm	Hgd* CAL. × 10^{-4}	HgA† CAL. × 10^{-4}	"DELAYED H.P." HgA-Hgd. CAL. × 10^{-4}	$\frac{Hgd}{Tm}$ CAL. × 10^{-4}	$\frac{HgA}{Tm}$ CAL. × 10^{-4}
		gm.					
Nitrogen:	2/19— 2:40 p.m.	464	17.9	23.3	5.4	3.86	5.02
		464	17.8	20.5	2.8	3.86	4.33
		1016	27.8	32.0	4.2	2.74	3.15
	2:55 p.m.	1531	45.9	56.4	10.5	2.99	3.25
		528	16.8	16.5	−0.3	3.16	3.16
	8:13 p.m.	553	14.5	12.1	−2.4	2.65	2.19
		1348	26.5	23.5	−3.0	1.97	1.74
		518	19.1	14.5	4.6	3.68	2.80
	2/20— 2:40 p.m.	440	5.6	9.2	3.6	1.27	2.09
		1011	25.5	27.5	2.0	2.55	2.72
		2629	60.0	74.8	14.8	2.27	2.85
		2513	62.8	83.5	20.7	2.50	3.33
						(12) 2.79	3.05
Oxygen-plenty:	2/19—12:30 p.m.	2171	73.2	100.2	27.0	3.37	4.63
	10:00 p.m.	445	14.7	10.5	−4.2	3.32	2.36
		802	17.6	19.9	2.3	2.19	2.48
		1309	30.3	43.5	13.2	2.30	3.32
		386	7.9	6.6	−1.3	2.05	1.71
		1660	34.7	48.3	13.6	2.08	4.91
		2651	55.4	92.2	36.8	2.10	3.48
		1346	25.5	35.6	10.1	1.90	2.64
	11:05 p.m.	1906	31.4	52.2	20.8	1.65	2.74
	2/20—10:25 a.m.	1728	29.6	66.6	37.0	1.71	3.85
		526	13.8	21.5	7.7	2.63	4.09
	11:18 a.m.	905	19.2	57.8	38.6	2.11	6.49
		2942	65.0	185.4	120.4	2.21	6.30
		1330	25.5	39.5	14.0	1.92	2.97
		488	12.7	24.2	11.5	2.60	4.94
						(15) 2.28	3.79

* Hgd is calculated here in the same way as in table 2 above.

† HgA is the product of the constant, $7.49 \cdot 10^{-4}$, found in table 2, and the total area of each of the several thermomyograms given under A.

latter cases the extreme deviations are 37 per cent of their mean and fully 200 per cent of each other, whereas tensions taken alone vary 760 per cent from one another. All the Hgd/T_m data are plotted in figure 4.

These observations then in the first place simply confirm the well known doctrine of the A. V. Hill-Meyerhof school, namely, that in the isometric twitch of isolated skeletal muscle heat production tends to bear a constant relation to tension exerted by the muscle.

In the second place, however, this study brings out the fact that the tendency for the H/T ratio to be constant is only a statistical one and the

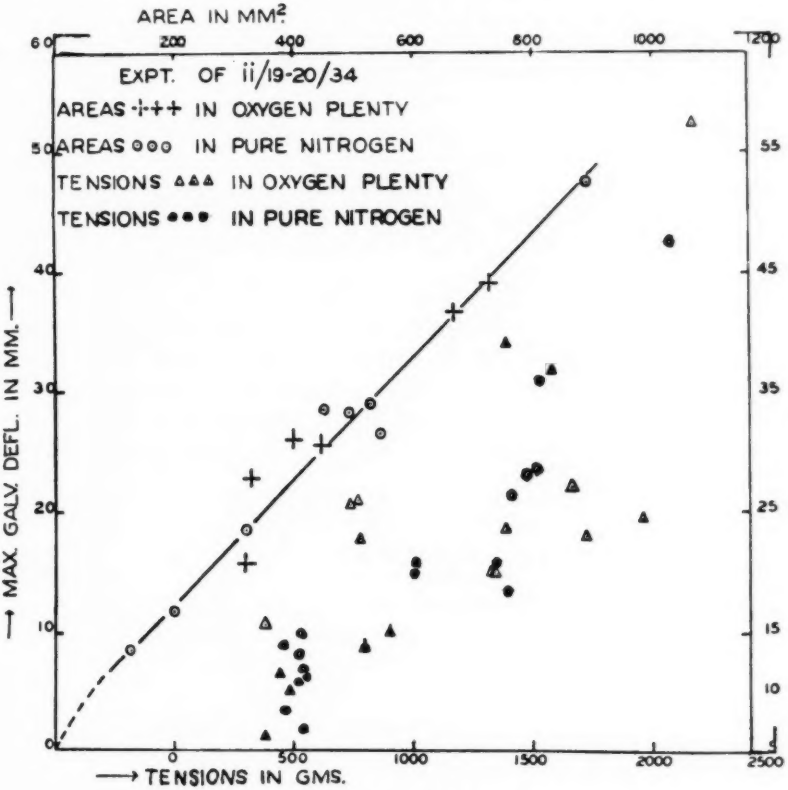

Fig. 4. See text for explanation.

deviations from the mean ratio in a number of observations on the same muscle and under the most constant of external conditions may be of very considerable magnitude, so great indeed that they no longer can be ascribed to experimental error. Rather must they be due to chance variations in the internal factors concerned with the contractile act. As in heart and smooth muscle, so the mechanism of contraction in frog's skeletal muscle appears to be very haphazardly prodigal in its energy expenditures. The

mere obligate minimal quantity required for the tension set up is very rarely doled out, and the recovery process is not always promptly carried out to completion and at no time with any great precision or neatness.

SUMMARY

1. A description and proof of the validity of a method for measuring heat liberated during the twitch of isolated muscle is given, which is based on the proposition that the total area inscribed by the galvanometer deflection-time curve of the thermomyogram and its appropriate base line, under given conditions, is of itself a measure of the total heat production concerned immediately with the twitch, and may more easily be measured by planimeter than by the method of harmonic analysis.

2. Reports of typical experiments on gastrocnemius, sartorius and rectus abdominalis muscles of *Rana pipiens* with final results and their analysis are given. It is shown that, while in a broad way the ratio of heat production to tension change tends to fluctuate around a constant value, yet under the most constant conditions many of the observed individual deviations from the mean are too great to fall within the limits of experimental error.

3. A brief discussion of the significance of these great deviations from the mean H/T value follows, from which the inference is drawn that the relations of input to output of energy in frog skeletal muscle, as in other kinds of muscle, are more like the input and output relations of a mill-race and the variable stream that feeds it than any one of the more efficient mechanical devices made by man.

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THE EFFECT OF OXYGEN ON MAN AT PRESSURES FROM 1 TO 4 ATMOSPHERES[1]

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This paper presents experimental data showing the tolerance of man to oxygen at pressures of 1, 2, 3 and 4 atmospheres.

METHODS AND APPARATUS. Healthy men between the ages of 22 and 40 served as subjects. They breathed oxygen once a week under the following conditions:

Group 1. The subject sat in a chair and breathed commercial oxygen through a mask connected with a Benedict apparatus. For pressures in excess of 1 atmosphere, the large pressure chamber described by Thomson, Yaglou and Van Woert (1932) was used. The analysis of oxygen from the cylinders showed < 0.5 per cent residual gas which was largely argon.

Group 2. The subject reclined on a cot, and with his head placed in a helmet breathed commercial oxygen at atmospheric pressure. The oxygen circulated through a closed system consisting of the helmet, a 30 liter spirometer, a cooling coil and a canister of soda lime. The temperature in the helmet was maintained at approximately 22°C. and the humidity at 40 per cent.

In the calculations for oxygen consumption a correction was made for the dissolved nitrogen eliminated from the body. The correction for the first 5-minute period (following a preliminary lung rinsing period of 5 minutes) was approximately 10 per cent of the basal oxygen consumption rate, and for the first 20 minutes about 5 per cent. For example, subject 1, with a basal rate of 214 cc. per minute, actually eliminated an average of 20 cc. of nitrogen during the first 5 minutes and an average of 10 cc. per minute for the 20-minute period. However, as the experiment progressed, smaller quantities of nitrogen were eliminated from the body, and after the first hour the correction became negligible.

Group 3. The subject sat in a chair and breathed oxygen through a mask at atmospheric pressure as in group 1. At intervals of 15 minutes, tests were made for neuromuscular coördination (eye-hand coördination)

[1] This research was aided by the Miriam Smith Rand Fund.

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and for the rapidity of response to a sound and light signal. The tests were conducted by means of a pursuit meter (Miles, 1921) which recorded a numerical score indicating the degree of incoördination and the time of response to the sound and light signal.

EXPERIMENTAL RESULTS. *Group 1.* The subjects breathed oxygen at 1, 2, 3 and 4 atmospheres.

At a pressure of 1 atmosphere, 10 individuals breathed oxygen from 2 to 4 hours. The only abnormal changes appeared in subject 10 who at the beginning of the 4th hour developed an increased respiratory rate (fig. 1, A *2*). In an experiment of 4 hours' duration with subject 10, a rise

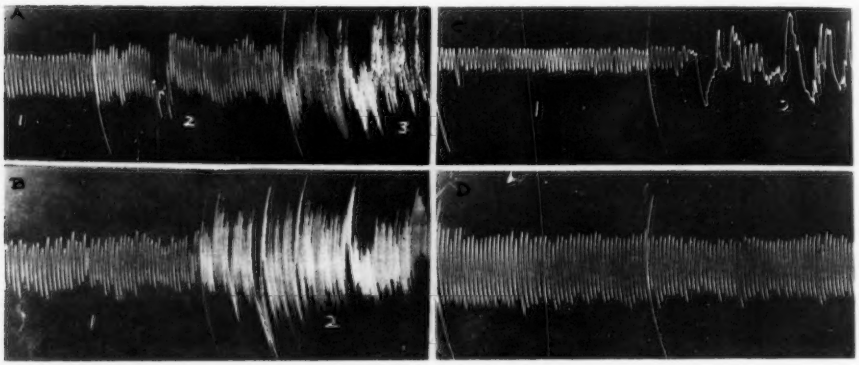

Fig. 1. A. Respiration of subject 10 breathing oxygen. *1*, during first 3 hours (resting); *2*, beginning of 4th hour (resting); *3*, beginning of 4th hour (running pursuit meter).

B. Respiration of subject 10 breathing oxygen during pursuit meter tests. *1*, during first $3\frac{1}{2}$ hours; *2*, following $3\frac{1}{2}$ hours.

C. Respiration of subject 11 breathing oxygen during pursuit meter tests. *1*, during first 2 hours; *2*, after the 2nd hour.

D. Control. Respiration of subject 11 breathing air during pursuit meter tests at the end of the 3rd hour.

in blood pressure and an increase in pulse rate occurred prior to the changes in respiration. During the 4th hour both the rate and the depth of respiration increased with alternate periods of rapid, shallow breathing.

At 2 atmospheres' pressure, 3 subjects breathed oxygen for 3 hours without abnormal subjective symptoms or changes in blood pressure and leucocyte count.

At 3 atmospheres' pressure, 4 subjects breathed oxygen for 2 hours. The only change observed was a slight increase in the white cells of the blood without a significant variation in the differential count. Subjective reactions were normal.

At 4 atmospheres' pressure, 2 individuals inhaled oxygen for approximately 45 minutes. Subject 10, after breathing oxygen 43 minutes without symptoms, suddenly experienced a transient syncope associated with blanching of the face, sweating of the hands, absence of radial pulse, and a

TABLE 1

Ninety-six to 99 per cent oxygen inhalation. Subject lying on a cot; 1 atmosphere pressure

SUBJECT	DURATION (MINUTES)	RESPIRATORY RATE		PULSE RATE		BLOOD PRESSURE		LEUCOCYTE COUNT	
		Start	Finish	Start	Finish	Start	Finish	Start	Finish
1	80	17*	17	72*	58	120/84*		6,000	6,900
	180	14*	16	81*	53	124/72*	122/76	11,900	8,300
	180	14*	16	88*	66	130/86*	130/98	7,200	6,150
	80	16	16					6,100	6,900
	160	18*	16	76*	66	116/70*	108/78	4,000	4,300
	240	14*	18	84*	56	114/70*	114/80	4,800	5,400
2	120	12	12					6,800	8,200
	180	13*	13	79*	70	124/72*	122/76	11,000	10,000
	180	13*	13	84*	68	128/80*	124/80	6,100	7,800
	160	18*	13	96*	62	130/78*	118/78	7,900	6,600
	160	12*	11	84*	74	120/80*	114/80	6,700	7,100
	160	18*	12	79*	66	112/76*	120/80	7,200	7,900
	240	13	18	84	68	114/70*	116/84	6,900	11,000
3	120	16*	15	62*	58	126/82*	118/88	9,900	9,000
	160	19*	14	76*	58	126/76*	114/78	8,400	6,800
	160	16*	14	72*	57	124/60*	130/84	7,500	8,400
	235	13	13	84*	61	120/80*	124/94	9,800	12,500
4	160	16*	18	84*	72	118/74*	116/78	5,900	5,800
	180	17*	18	82*	72	122/78*	116/78	8,200	6,500
	160	20*	16	92*	66	138/84*	124/88	6,100	6,500
	160	16*	16	80*	60	134/80*	122/82	6,600	7,600
	240	18	15	94*	60	124/84*	122/88	8,300	7,300
9	125	14	Dyspnea	60*	66	120/66*	136/66	10,400	9,400
10†	240	20	16	68	60	128/68	108/80	7,300	21,200

Next day, W.B.C. 14,850. Neutrophils 50 per cent, lymphocytes 32 per cent, eosinophils 13 per cent, monocytes 5 per cent.

* Subject breathing air.

† Fatigue after the experiment with symptoms of bronchial irritation.

fall in blood pressure. With the inhalation of air, complete recovery followed immediately. Subject 1, after breathing oxygen 43 minutes, felt well. No significant changes occurred in the pulse rate, the blood pressure, the respiratory rate or the minute volume. During the 44th minute,

however, a temporary twitching of the left eyebrow was observed. The subject then uttered a short cry, and developed a convulsion, characterized in the beginning by violent tonic movements, and then by clonic contractions of the muscles of the head, trunk, and extremities. Cyanosis did not occur; sphincter control was maintained. With the subsidence of the convulsive movements the subject was in a stuporous condition for about 13 minutes. During this period the face turned an ashen grey color, beads of perspiration appeared on the forehead, and breathing became

TABLE 2

Oxygen consumption during inhalation of 97 per cent oxygen at 1 atmosphere of pressure

*DURATION OF EXPOSURE (MINUTES)..				20	40	60	80	120	160	180	240
TIME INTERVALS (MINUTES).....				20	20	20	20	40	40	20	60
Subject	Calculated basal rate	Experiment	Rest period (minutes)	Oxygen consumption (cc./min.)							
1	214	1	20	257	234	236	245	247	244	223	
		2	60	245	234	234	238	239	225	223	
		3	45	240	208	204	217	196	203	209	
2	249	1	10	287	276	266	278		281	281	
		2	20	293	256	259	247	256		259	
		3	60	317	284	279			277	263	
		4	50	325	279	278	267	264	264	264	283
3	266	1	15	304	283	298	284	286			
		2	18	343	289	291	280	275	284		
		3	50	334	288		273		267	267	287
4	221	1	20	274	236	238	231	234	231		
		2	30	272	236	231	225	229	235	224	
		3	60	240	239	233	228		228		
		4	15	265	236	218		229	221	221	228

* Following a preliminary lung rinsing period of 5 minutes.

stertorous. When he regained consciousness 20 minutes after the onset of the convulsion, the subject thought that he had fallen asleep. Recovery was complete.

Group 2. Six subjects reclining on a cot, breathed oxygen in a helmet at 1 atmosphere pressure for periods of 1 to 4 hours (table 1). An increase in the leucocyte count occurred in 3 subjects after they breathed oxygen 4 hours. The only symptoms of pulmonary irritation were experienced by subject 10. He felt well during the inhalation of oxygen, but when he again breathed air, he was conscious of substernal discomfort and coughed occa-

sionally during the first half hour. The leucocyte count increased from 7,300 to 21,200 cells. On the following day the leucocyte count decreased to 14,850 cells with a differential count of 13 per cent eosinophils. With the decrease in the blood count the pulmonary symptoms disappeared and only a moderate degree of fatigue remained. Subsequent 4 hour exposures to oxygen did not produce comparable changes in this subject.

TABLE 3

"Pursuit meter" tests. Subject sitting in a chair. Oxygen pressure 1 atmosphere

SUBJECT	EXPOSURE	COMMENT
	hours	
9	3	Hyperpnea and irregular respiration during the last hour while performing tasks
10 (a)	2.75	Although the score recorded by the "pursuit meter" was constant, greater effort was required after 10 5 minutes and hyperpnea accompanied each succeeding task Blood pressure, 1st hour—118/78 Blood pressure, end of experiment—152/110
(b)	3.7	Score constant but greater effort required after 3½ hours. Hyperpnea with each succeeding task. (Fig. 1, B *2*.) No change in blood pressure
(c)	4	After 3 hours an increase (spontaneous) in respiratory rate occurred (fig. 1, A *2*). Then the 1st "pursuit meter" test of the experiment was made and the subject's reaction is illustrated by figure 1, A *3* Coördination and attention impaired Blood pressure (start, 11:00)—126/86 Blood pressure at 12:00—134/102 Blood pressure (termination of experiment)—140/106
11	3.3	After 1 hour a definite impairment in coördination and attention occurred. Subjectively the task seemed more difficult. After 2 hours an irregular type of respiration developed. (Fig. 1, C *2*.) Compare with control, end of 3rd hour (fig. 1, D)
12	4.3	No changes in coördination or respiration

The only other abnormal reaction was observed in subject 9, who developed dyspnea and coarse tremors of the hands after breathing oxygen for 2 hours.

The values for oxygen consumption of 4 individuals (group 2), 3 hours following the morning meal, are shown in table 2. After the first 20 minutes the utilization of oxygen remains constant for the remainder of the

experimental period. The high values of the first 20 minutes are not related to the duration of the preliminary rest period, nor, on account of their short duration, can they be ascribed to the effect of the morning meal. A large part of the increase occurs during the first 5 or 10 minutes. The oxygen going into physical solution would account for only a small part of this increase, and the oxygen coming into equilibrium with the intestinal gases should require a longer period of time (McIver, Redfieldt and Benedict, 1926).

Group 3. Tests were performed with the pursuit meter while breathing oxygen at 1 atmosphere pressure. Three subjects were examined during periods of 3 to 4 hours and the results are given in table 3. In subject 11, impairment in coördination and the power of attention occurred after the first hour, and during the third hour a slow, irregular type of respiration developed (fig. 1, C *2*). In subjects 9 and 10, after the third hour of exposure, coördination and attention were maintained only with great effort. The hyperpnea and the increase in blood pressure are regarded as effort responses. Subject 10 (c) breathed oxygen for 3 hours without making tests on the pursuit meter. Following a spontaneous increase in respiratory rate (fig. 1, A *2*) a pursuit meter test was made, and the respiratory response is shown in figure 1, A *3*.

Discussion of results. *The toxic effects of oxygen.* 1. *On the nervous system.* The toxic effects of oxygen are referable mainly to the nervous system. At 4 atmospheres' pressure, the oxygen in physical solution (approximately 7 vols. per cent) is sufficient to satisfy the tissue requirements (Behnke, Shaw, Shilling, Thomson and Messer, 1934) and at this pressure the striking effects of oxygen are seen. Subject 10 experienced transient syncope and a fall in blood pressure but recovered immediately when air was substituted for oxygen. Subject 1 developed a generalized convulsive seizure after 45 minutes' exposure. While no similar seizures have been reported in the literature for man, they have been produced in rats, cats, goats, and monkeys at 4 atmospheres' pressure. The complete recovery when air is substituted for oxygen is characteristic of the toxic action of oxygen at 4 atmospheres' pressure (Shaw et al., 1934) and indicates that oxygen either acts directly on the nervous system or produces rapidly reversible chemical changes.

At a pressure of 1 atmosphere, oxygen during a period of several hours may bring about an impairment in neuromuscular coördination or an increased effort to maintain coördination (table 2). Subject 12, however, breathed oxygen 4.3 hours without showing changes in respiration or neuromuscular coördination. Moreover, while pursuit meter tests were not made at 2 and 3 atmospheres' pressure, the individuals who breathed oxygen at these pressures did not report abnormal subjective symptoms.

The hyperpnea appearing in subjects 9 and 10 during the tests on the pursuit meter, is interpreted as an effort response. In the intervals between tests, the marked hyperpnea subsided. However, subjects 9 and 10 after 3 and 4 hour exposures to oxygen in later experiments (without the pursuit meter) showed a spontaneous increase in respiratory rate. Corroboration of this finding on additional subjects is necessary before conclusions can be drawn, but it suggests an early manifestation of the effect of oxygen on the nervous system. In anesthetized dogs, oxygen at 4 atmospheres' pressure acts on the neurorespiratory mechanism to produce first, convulsive breathing, and then paralysis of respiration (Shaw et al., 1934). In man, it is conceivable that prolonged exposure to oxygen at atmospheric pressure may affect the nervous centers controlling respiration by increasing their sensitivity to carbon dioxide (fig. 1, A *2*). The shallow breathing following increased pulmonary ventilation in subject 10 (experimental results, group 1) may be the result of lowered alveolar carbon dioxide tension, or fatigue of the respiratory centers induced by oxygen. It is interesting to note that a lowering of the carbon dioxide tension in the blood would render the action of oxygen on the nervous system less effective by slowing the cerebral circulation (Wolff and Lennox, 1930).

2. *Pulmonary damage.* The pulmonary changes are not impressive, and are of minor importance in comparison with the changes referable to the nervous system. At 4 atmospheres' pressure, after 45 minutes' exposure, symptoms indicative of pulmonary damage did not occur in two subjects. At 3 atmospheres' pressure, incipient pulmonary changes may be responsible for the increased leucocyte count, but symptoms of pulmonary irritation (cough, pain in the chest) were absent. At 1 atmosphere pressure, the only indication of lung damage occurred in subject 10 who developed a cough, leucocytosis and substernal pain after he breathed oxygen for a period of 4 hours in the recumbent position. Subsequent exposures, however, with the subject sitting in a chair, have not produced comparable changes.

Increased acidity as a cause of oxygen poisoning. Gesell (1922), Bean (1932), Campbell (1929–1930), and Hill (1933) stress the rôle of increased tissue acidity due to carbon dioxide retention as a factor in the production of the convulsions of oxygen poisoning. Increased tissue acidity resulting from exposure to oxygen is ruled out by the constancy of the respiratory minute volume at 1, 2, 3 and 4 atmospheres' pressure. While there are minor fluctuations in minute volume, there is no consistent tendency toward an increase as the oxygen pressure is raised. It is true that carbon dioxide increases the effectiveness of a given oxygen pressure. For example, carbon dioxide added to oxygen produces convulsions sooner and at a lower pressure than oxygen acting alone (Hill, 1933). However, it cannot

be inferred from this statement that carbon dioxide is responsible for the convulsions since they occur with normal or even subnormal alveolar carbon dioxide tensions if the oxygen tension is sufficiently high (Shaw et al., 1934).

SUMMARY AND CONCLUSIONS

The mental and physical effects induced by breathing 96 to 99 per cent oxygen were studied in man at pressures of 1, 2, 3 and 4 atmospheres.

Healthy men between the ages of 22 and 40 can breathe pure oxygen with comparative safety as follows: 4 hours at 1 atmosphere; 3 hours at 2 atmospheres; 2 hours at 3 atmospheres.

The symptoms induced by oxygen are referable mainly to the nervous system. At 4 atmospheres' pressure convulsions occurred in one subject and syncope in another after exposures of approximately 45 minutes. Only 2 subjects were tested at this pressure. At a pressure of 1 atmosphere, impaired neuromuscular coördination and the power of attention or an increased effort to maintain these functions occurred after 1 to 3 hours' exposure in 3 out of 4 subjects tested. Hyperpnea occurred after 3 hours' exposure to oxygen in 2 out of 11 subjects.

The irritative effect of oxygen on the lungs was noted in only 1 subject on a single occasion. The symptoms were substernal pain, dry cough, and a high leucocyte count. The subjects exposed to the higher oxygen pressures were singularly free from pulmonary symptoms.

Oxygen consumption is high during the first 20 minutes, and then decreases to a level which is constant for periods up to 4 hours at atmospheric pressure.

In all of the oxygen exposures, the blood pressure, the respiratory rate, and the minute volume (with two exceptions) were constant at 1, 2, 3 and 4 atmospheres' pressure.

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THE TYROSINE CONTENT OF TISSUES AFTER INTRAVENOUS INJECTION

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A rapid disappearance of the amino-acid tyrosine from the blood stream was found by King and Rapport (1933) after injecting as much as 5 grams of the amino-acid intravenously into the external jugular vein. This disappearance was found to be more rapid in the case of tyrosine than for equivalent amounts of glycine and alanine (cf. also Van Slyke and Meyer, 1913). There was no appreciable increase in the urea formation in the blood, hence it appeared that the disappearance was not associated with breakdown via deaminization. Moreover, no more than 5 per cent of the total tyrosine injected could be recovered in the urine in 24 hours as phenols. Therefore, it appeared probable to us that the tyrosine must either be deposited in the various tissues of the body as such, or that it must be rapidly changed in structure with the destruction of the phenol group. We have therefore attempted to trace the fate of the remaining 95 per cent of the tyrosine by analysis of various tissues of the body for phenols.

EXPERIMENTAL METHODS. Dogs weighing between 9.5 and 11.0 kgm. were used for this investigation. Since each dog was to be used for one acute experiment, no constant diets or starvation periods were considered necessary. Each dog was given a preliminary injection subcutaneously of 0.8 cc. of 3 per cent morphine sulphate and anesthesia obtained by 1.5 cc. per kilo of body weight of a mixture of chloretone, ether and petrolatum (50 gm. chloretone; 150 gm. petrolatum; 350 gm. ether). This corresponds to 150 mgm. chloretone per kilo, and was injected intraperitoneally 15 minutes following the administration of the morphine sulphate. Deep anesthesia, sufficient for all surgical procedures, was obtained in about 30 minutes, and was maintained at least 8 hours or to the completion of the experiment.

Without regard for asepsis, but with strict precautions to prevent undue hemorrhage, one lobe of the thyroid gland, and the left adrenal were removed. Approximately 1 gram samples of various tissues were taken, namely, skeletal muscle (gluteus maximus or sterno-mastoid) by excision after ligature; pancreas by excision after ligature; uterus by excision after ligature; kidney by resection after applying mattress sutures; and liver and spleen by excision followed by hot cauterization of the cut surface.

Five grams of tyrosine, dissolved in N/1NaOH with a resulting pH of about 10.2, were injected into the left femoral vein over a period of two to three minutes. Ten cubic centimeter samples of blood were removed at various intervals after injection of the tyrosine from the right femoral or left external jugular vein. Samples of the respective tissues mentioned above were also taken in the manner previously described for each one.

In the blood samples, NH_2 nitrogen, phenols, and in some cases urea nitrogen were determined. Blood NH_2 nitrogen was determined by the method of Folin (1922), blood urea by the method of Folin and Wu (1919), and blood phenols by a modification of the methods of Folin and Marenzi (1929) (cf. King and Rapport, 1933).

The respective tissue samples were dried in a constant temperature electric oven maintained at 95°C. for 24 hours. Approximately 100 mgm. of dried tissue were taken for analysis, accurately weighed, and analyzed for phenols by the method of Folin and Marenzi (1929) without modification. Samples of bile were analyzed in a similar manner.

EXPERIMENTAL RESULTS. a. *Blood phenols.* The control blood in every experiment failed to show the presence of any phenols by this technique. After injection of 5 grams of tyrosine, definite tests for phenols could be obtained, the amount however rapidly decreasing in a very short time (fig. 1). With the exception of three experiments wherein the phenol content of the blood averaged about 200 mgm. per 100 cc. of blood, the amount found within five minutes after injection averaged about 60 mgm. per 100 cc., indicating a rapid disappearance of tyrosine from the blood.

b. *Blood NH_2 nitrogen.* Although the increase in NH_2 nitrogen in the blood was significant in every experiment, the amount was no greater in any experiment than what may be calculated to be present on the basis of the tyrosine found at that time. The rapid decrease in the amount within one hour after injection of the tyrosine is similar to the results found with the blood phenols.

In respect to the blood phenols and NH_2 nitrogen, the findings were somewhat higher than those observed by King and Rapport. The difference is probably to be attributed to the fact that in the present experiments the animals were anesthetized, this being the only way in which the technique differed.

c. *Blood urea nitrogen.* In a few of the experiments the blood urea nitrogen was determined, and the results confirmed the previous findings of King and Rapport, that the apparent disappearance of tyrosine from the blood was not associated with increased deaminization, there being no significant rise in the urea nitrogen after tyrosine injection.

d. *Tissue tyrosine.* Fifteen experiments were performed in which tissue analyses were made after tyrosine injection; also, a control experiment in which the procedure was identical with these except that tyrosine was not

administered. In five of the fifteen experiments, the following tissues were analyzed: skeletal muscle, liver, spleen, kidney, thyroid, pancreas and adrenal. In three of these five experiments, uterus was also analyzed. In the remaining ten experiments, not all of these tissues were analyzed in any one experiment. For lack of space, the data for each tissue in only the first five mentioned experiments are included in table 1. The results for the remaining experiments are in substantial agreement.

1. *Skeletal muscle.* Analyses of samples of skeletal muscle showed the surprising result that there was practically no change in the phenol content

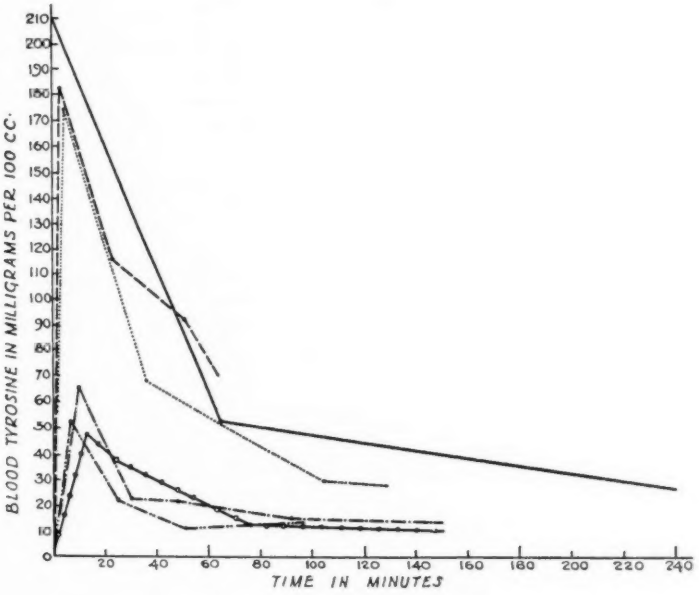

Fig. 1

before and after injection of tyrosine. In this respect, tyrosine appears to differ from other amino acids, such as glycine and alanine for example, which have been shown to be deposited in muscular tissue to some extent. The average increase in four experiments in which samples were taken at relatively the same time after injection, was 0.4 per cent one hour after injection, and 0.3 per cent two hours after injection. The greatest increase was 5.5 per cent in experiment 5. The apparent decrease in dog 9 (6.4 per cent) was perhaps due to the small size of the muscle used for analysis, which did not allow for accurate colorimetric comparison. Examples of the results are seen in table 1.

TABLE 1

TISSUE	DOG NO.	TYROSINE IN TISSUE						
		Before injection	Sample 1			Sample 2		
		Tyrosine	Time after injection	Tyrosine	Change	Time after injection	Tyrosine	Change
		per cent	*minutes*	*per cent*	*per cent*	*minutes*	*per cent*	*per cent*
Muscle	9	2.97	60	2.78	−6.4	120	3.04	+2.3
	12	3.04	60	3.03	−0.3	120	3.01	−1.0
	13	3.41	60	3.46	+1.4	120	3.44	+0.9
	14	3.23	60	3.26	+0.9	120	3.24	+0.3
	15	3.18	60	3.17	−0.3	120	3.16	−0.6
	Control	2.59		2.59	0.0			
Adrenal	9	1.72	173	2.03	+18.0			
	12	1.79	173	2.10	+17.6			
	13	1.95	173	2.06	+5.7			
	14	2.24	173	2.41	+7.6			
	15	2.10	173	2.32	+10.5			
	Control	2.04		2.00	−1.9			
Thyroid	9	3.61	60	4.26	+18.0			
	12	2.49	60	2.80	+12.5			
	13	2.94	60	3.25	+10.6			
	14	2.72	60	2.85	+4.6			
	15	Thyroidectomized						
	Control	3.82		3.80	−0.5			
Pancreas	9	3.63	60	4.13	+13.8	140	3.87	+9.1
	12	4.07	60	4.69	+15.2	140	4.54	+11.5
	13	3.42	60	3.89	+13.7	140	3.78	+10.5
	14	3.17	60	3.47	+9.5	140	3.42	+7.9
	15	3.19	60	3.68	+15.4	140	3.61	+13.2
	Control	3.64		3.60	−1.1			
Liver	9	2.58	90	2.77	+7.4	145	2.68	+3.9
	12	3.31	90	3.31	0.0	145	3.33	+0.6
	13	3.18	90	3.16	−0.6	145	3.10	−2.5
	14	2.98	90	3.04	+2.0	145	3.02	+1.3
	15	2.71	90	2.69	−0.7	145	2.71	0.0
	Control	2.87		2.91	+1.4			
Spleen	9	2.75	70	3.24	+17.4	140	3.10	+12.7
	12	3.27	70	3.28	+0.3	140	3.25	−0.9
	13	3.52	70	3.49	−0.9	140	3.48	−1.1
	14	2.86	70	2.84	−0.7	140	2.87	+0.4
	15	2.96	70	2.98	+0.7	140	2.98	+0.7
	Control	3.40		3.41	+0.3			
Uterus	13	2.93	110	2.75	−6.1	160	2.91	−0.7
	14	4.73	110	4.28	−9.5	160	4.47	−5.5
	15	2.86	110	2.88	+0.7	160	2.85	−0.3
	Control	3.08		3.00	−2.6			
Kidney	9	3.38	110	3.40	+0.6	180	3.31	−2.7
	12	3.38	110	3.47	+2.6	180	3.47	+2.6
	13	3.36	110	3.33	−0.9	180	3.29	−2.1
	14	3.11	110	3.11	0.0	180	3.12	+0.3
	15	3.81	110	3.83	+0.7	180	3.78	−0.8
	Control	3.19		3.15	−1.2			

2. *Spleen.* For the spleen, the average increase in four experiments was 0.0 per cent 69 minutes after injection; and 0.0 per cent after 140 minutes. The greatest increase, exclusive of experiment 9, was 0.7 per cent in dog 15. The large increase in experiment 9 (19 per cent) can possibly be attributed to the large amount of blood present in the sample not being washed out before drying for analysis.

3. *Kidney.* In the kidney, chiefly cortex, the average increase in five experiments was 0.6 per cent after 110 minutes, and 0.2 per cent after 180 minutes. The greatest increase was 2.6 per cent in dog 12.

4. *Uterus.* In two experiments, the phenol content in the uterus decreased after the injection. The samples were very small and accurate colorimetric comparison was impossible. Experiment 15, however, showed only 0.7 per cent increase 110 minutes after injection.

5. *Adrenal.* The average increase in the adrenal was 11.9 per cent when removed 173 minutes after injection of the tyrosine. The smallest increase was 5.7 per cent in dog 13; and the largest increase was 20.4 per cent in dog 2, 28 minutes after injection.

6. *Pancreas.* In the pancreas, the average increase of five experiments one hour after injection was 13.5 per cent; and two hours after injection was 10.4 per cent. The greatest increase was 37.1 per cent in dog 4, 5 minutes after injection, whereas the smallest increase was 5.8 per cent 5 hours after injection in dog 6.

7. *Thyroid.* The average increase in the tyrosine content of the thyroid in four experiments was 11.4 per cent. The greatest increase was 37.4 per cent, 2 minutes after injection in dog 1; and the smallest increase 1.9 per cent, 5 minutes after injection in dog 5. Dog 15 had previously been thyroidectomized, and in this dog the findings in the other tissues examined were not significantly different from those in the normal animals.

This is of interest in connection with the paper of King and Rapport, in which no appreciable difference in the rate of disappearance of tyrosine from the blood was observed as between normal and thyroidectomized dogs. In that communication, the following statement was therefore made: "it may be inferred that the thyroid is not primarily concerned with the rapid disappearance of blood tyrosine, though the probability that the thyroid may take up this amino acid is not of course ruled out." The present evidence indicates that the inference of King and Rapport must be modified, as the thyroid clearly takes up part of the tyrosine.

8. *Liver.* The liver varied in results depending upon whether the sample was washed of its blood content before drying for analysis. The average increase in five experiments where the blood was washed out, was 1.6 per cent after 90 minutes. In dog 1 an increase of 39.5 per cent was found, 5 minutes after injection when the blood was not removed from the sample.

9. *Bile.* No significant amounts of phenols were found in any samples of bile taken for analysis.

A comparison of the tyrosine uptakes by the individual tissues in two representative experiments has been made in figure 2.

The control experiment indicates that the phenol content is constant for different samples of the same tissue, even though successive samples are

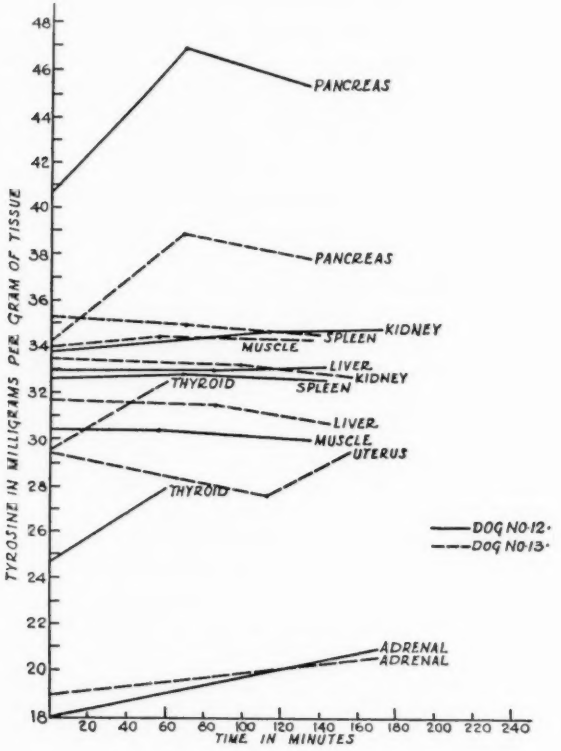

Fig. 2

taken at some distance from the site of the other samples, and in the case of the thyroid, adrenal and kidney from the right and left organs. Two samples of each tissue were analyzed, the second samples being removed after all surgical procedures, required before injection of tyrosine, were completed.

When all samples were washed of any blood contained in them, the only significant increases in phenol content were found in the adrenal, the pan-

creas, and the thyroid. This is of interest because all are glands of internal secretion and their hormones appear from their chemical structures to have some relation to tyrosine. Other organs and tissues not known to elaborate definite hormones related to tyrosine have failed to show any significant increase in phenol content.

An attempt was made to estimate approximately the total amount of tyrosine taken up by the tissues which did show an increase. On the basis of the dried weight of these tissues, it was calculated that not more than five per cent of the tyrosine administered could be so accounted for. Since it has been previously shown that only about five per cent likewise could be accounted for as increased phenols in the urine for 24 hours after injection, it is evident that the fate of approximately 90 per cent of the tyrosine administered is as yet unknown.

CONCLUSIONS

1. After the injection intravenously of 5 grams of tyrosine into dogs, there is a rapid disappearance of the amino acid from the blood.

2. The slight, if any, rise in urea nitrogen of the blood does not account for any appreciable amount of the tyrosine being deaminized.

3. Of the organs studied, the thyroid, adrenals and pancreas are the only tissues significantly to increase in phenol content after the injection of tyrosine.

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METHYLENE BLUE AND ANOXEMIA

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In the last few years there have been many papers on the use of methylene blue in cases of cyanide and carbon monoxide poisoning. While everyone agrees that it is valuable in the treatment of cyanide poisoning, there is an ever-increasing number of authors who believe that it is not only useless but actually contraindicated in cases of poisoning from carbon monoxide. It was originally propounded that methylene blue was able to restore or substitute for tissue oxidases which had been neutralized or destroyed by the poison in each case. This theory has given way to another which has it that the methylene blue forms methemoglobin. Methemoglobin has a great affinity for cyanides and, providing the patient can spare some of his hemoglobin, this is a good way to "extract" the cyanide from his more vital tissues. Carbon monoxide kills an intact animal by asphyxiation from hemoglobin deficit. Methylene blue, by changing still more of the hemoglobin into methemoglobin, which also has no oxygen carrying power, would seem to "synergize" the lethal effects of carbon monoxide. It was reasoned that a crucial test of this last hypothesis might be made by using simple anoxemia. This paper presents data showing that methylene blue does not prevent but seems to enhance the effects of oxygen want.

METHOD. In each of two experiments litter mate pups were divided into three groups: 1, an experimental animal which was injected intravenously with 10 to 16 mgm. of methylene blue per kilo body weight and placed in a steel respiratory tank, where various degrees of anoxemia were produced by partially decompressing while constant ventilation prevented carbon dioxide accumulation; 2, an anoxemia control which was injected with a corresponding volume of physiological saline and placed in the tank along with the experimental animal, being subjected to the same degree of anoxemia, and 3, a methylene blue control which was injected with the same dose of methylene blue used in the experimental and left at atmospheric pressure. The anoxemia was begun immediately after injection.

In the second part of the experiments white rats were used. Here methylene blue and saline were injected intraperitoneally. In each given experiment the animals were divided into three groups (experimental, anoxemia control and methylene blue control corresponding to the groups of pups) of five rats each. It was found necessary to run the control groups every

time as the results of one experiment were not directly transferable to a succeeding one. Care was taken to have the same weight and sex distribution in each group. The experimental procedure was the same as for the pups with varying doses of methylene blue and varying degrees of anoxemia, except that in some experiments 30 minutes were allowed to elapse between the injections and the beginning of the anoxemia, in case there was a latent period between the time of injection and methemoglobin formation (see notes in table 1 B). Such superficial observations as could be made through the glass window of the tank were noted, but the time and number

TABLE 1

EXPERIMENT NUMBER	METHYLENE BLUE DOSAGE	ANOXEMIA DEGREE	TIME: INJECTION TO ANOXEMIA	EXPERIMENTALS			ANOXEMIA CONTROLS			METHYLENE BLUE CONTROLS		
				Total number	Number dead	Per cent fatality	Total number	Number dead	Per cent fatality	Total number	Number dead	Per cent fatality
A. Pups												
	mgm. per kilo	*per cent*	*minutes*									
1	10	6.0	None	1	1	100	1	0	0	1	0	0
	6	6.0	None	1	1	100						
2	16	6.7	None	1	1	100	1	0	0	1	0	0
B. Rats												
1	132	11.0	None	5	5	100	5	0	0	5	3	60
2	35	10.0	None	5	1	20	5	1	20	5	0	0
3	50	5.5	None	5	5	100	5	4	80	5	0	0
4	50	6.7	None	5	3	60	5	2	40	5	0	0
5	54	6.7	30	5	5	100	5	0	0	5	0	0
6	50	6.7	30	5	4	80	5	1	20	5	0	0
7	43	6.0	30	5	5	100	5	2	40	5	0	0
Totals.....				35	28	80	35	10	29	35	3	9

of deaths in each experiment were taken as the measure of the combined effects of methylene blue and anoxemia.

RESULTS. The results are summarized in table 1. Of special interest are the facts: 1. All three of the pups in the two experiments receiving methylene blue and anoxemia died while none in the anoxemia or methylene blue control groups died. 2. In all but one experiment, more rats in the experimental group died than in the two control groups combined. The total percentage of fatality for the various dosages and various degrees of anoxemia were: experimentals, 80; anoxemia controls, 29, and methylene blue controls, 9. Correcting the 80 per cent fatality among the experi-

mentals for the 29 and 9 per cent caused respectively by anoxemia and methylene blue alone, methylene blue seems to be responsible under anoxemic conditions for at least a 42 per cent fatality. The fatalities in the methylene blue controls were all from the one huge dosage group which, of course, was necessary to establish an upper extreme of dosage safety. There were no significant differences in the time of death in the experimental and anoxemic control groups, even though the percentage fatality was differential. The rats which were killed by huge doses of methylene blue alone lived five times as long as the ones receiving the same dose of methylene blue and subjected to anoxemia.

There was a tendency for the anoxemia control rats to remain lively and curious while the experimentals became prostrated early. However, this was not nearly so significant as in the case of the pups, for the experimental rats were not so much more depressed than the methylene blue controls outside. In the case of the pups there was a tendency for early convulsions in the experimentals, the convulsions becoming fewer and fewer and the animals going finally into coma before death. In the anoxemia control pups there were few convulsions early, but later there were more convulsions; and one of the anoxemia controls was in convulsions at the end of the experiment when removed from the tank, after the experimental had just died in coma. In one experiment in which respiratory rates were checked, it was found that at corresponding times the respiratory rates of the experimental pup were 75, 71, 54 and 54 per minute and for the anoxemia control, 46, 25, 37 and 53.

Discussion. The dosage of methylene blue in the rat experiments was admittedly high. When coupled with the experiments on pups, however, it seemed significant. In experiments B 1 and 2 (table 1), extremes of dose range were considered established. All of the other experiments, therefore, were with intermediate doses which would give positive results.

With asphyxia or anoxemia, although there may be the normal amount of hemoglobin present, the margin of safety against oxygen want may be exceeded because the hemoglobin is not an efficient carrier of oxygen when they are in equilibrium at a low pressure. Although the hemoglobin is saturated, the oxygen content is low because of the low oxygen value operating in the mass action involved in the hemoglobin-oxygen combination. If a part of the hemoglobin is previously changed into methemoglobin, which is useless for oxygen transportation, it is evident that symptoms of insufficient oxygen delivery will occur sooner, and this is what these experiments show when methylene blue reacts with hemoglobin to form methemoglobin.

As confirmatory evidence for a low margin of safety so far as the hemoglobin was concerned, was the more rapid breathing in the experimental (hemoglobin-poor) animals. These animals had respiratory rates averag-

ing almost twice that of the anoxemia controls. They also showed "asphyxial convulsions" early in the experiments, which proceeded into profound depression before these convulsions appeared in the anoxemia controls—they were asphyxiated more rapidly.

While it is true that cyanides and carbon monoxide can neutralize or destroy tissue oxidases, the concentrations of these substances must be much higher than the lethal dose in the intact animal. This is particularly true of carbon monoxide. Death of an animal from this poison, therefore, must be through some other mechanism. Methylene blue readily forms methemoglobin when added to hemoglobin. This cuts down on the oxygen carrying capacity of the blood and reduces the margin of safety against oxygen want. Because of this normally rather wide margin of safety, the loss of part of the hemoglobin is clinically justifiable if by so doing some desired end is accomplished without subjecting a patient at the same time to the dangers of oxygen want. Such is the case in cyanide poisoning. Here the important thing is to draw the cyanide out of the more vital tissues of the animal, and methemoglobin is able to do it effectively while there is no danger of symptoms of anoxemia, providing the hemoglobin has not been reduced below the margin of safety from some other cause.

Carbon monoxide, as has been fully shown by others, kills an intact animal by asphyxiation through its action of changing oxygen-carrying hemoglobin into non-oxygen-carrying carbon monoxide hemoglobin. If on top of a sublethal exposure to carbon monoxide some more of the hemoglobin is changed into non-oxygen-carrying methemoglobin, the animal will be more, rather than less, likely to become asphyxiated. The effect of the two will be additive.

If methylene blue has any value in carbon monoxide poisoning, as has been reported clinically—but in cases where the multiplicity of the therapeutic measures themselves obviates all controls—the most that can be said is that it does not act by restoring tissue oxidases or by offsetting the effect of carbon monoxide on the blood or tissues. It must function in some roundabout way not yet discovered and difficult to conceive of. From these experiments we feel forced to join the ranks of those who deem methylene blue contraindicated in cases of carbon monoxide poisoning or any other condition in which the margin of safety of hemoglobin concentration has already been encroached upon.

SUMMARY

Methylene blue augments the symptoms of anoxemia, substantiating its contraindication in carbon monoxide poisoning.

EFFECTS OF THYROPARATHYROIDECTOMY AND CARBOHYDRATE INTAKE ON THE ACTION OF ANTERIOR PITUITARY EXTRACTS

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There is good evidence that the pituitary gland may increase metabolism indirectly, by way of the thyroid gland. Foster and Smith (1) and Smith (2) found that anterior pituitary transplants raised the low metabolism of hypophysectomized rats and caused repair of the associated thyroid atrophy. It has since been established that thyroid hypertrophy and hyperplasia (3), (4) and temporary increases of the metabolism (5), (6) can be produced in various animals by means of anterior pituitary extracts.

Whether the calorigenic action of pituitary preparations is entirely dependent on the presence of the thyroid gland is not so clear. In the thyroparathyroidectomized dog, Bueno and Barnes (7) obtained no calorigenic effect with Loeb's acid extract, while the writer did obtain this effect with Teel's partly purified growth preparation of beef anterior lobes (8). Using the alkaline extract of Evans and Simpson, Houssay and Artundo (9) found the increase of metabolism in thyroidectomized dogs one-fifth to three-fourths as large as in normal dogs. In the following studies this point is investigated further, with special reference to the rôle of accessory thyroid tissue, including the intrapericardial thyroid nodules described by Swarts and Thompson (10) and Davis (11). Whether thyroparathyroidectomy alters the remarkable effect of the beef anterior lobe preparation on nitrogen and water metabolism (12) was also studied. Since the calorigenic effect of the preparation, like that of thyroxin (13), is accompanied by a fall in the basal respiratory quotient, due to increased fat oxidation, it was likewise of interest to determine whether an increase of the carbohydrate supply would prevent this, or whether there is preferential oxidation of fat. Anselmino and Hoffmann (14) separated from anterior pituitary lobes a fraction which is said to stimulate fat metabolism.

METHOD. Large female dogs were placed on diets of beef heart, cracker meal and bone meal, prepared as described previously (12). The daily ration was constant and was consumed completely. Averages of many analyses are given at the foot of the tables. After a control period, one 50 cc. dose of the Parke-Davis preparation "Antuitrin-G" was injected

subcutaneously in 10 to 15 cc. portions. Daily determinations of the water intake, urine volume, weight, urine nitrogen, and, when possible, of the basal metabolism were then continued until the control values were reached again. The injected preparation consists of the fraction which sodium sulfate precipitates from partially neutralized alkaline extracts of beef anterior lobes (15), (16). Fifty cubic centimeters represented 25 grams of anterior lobes. An alkaline extract of whole pituitaries of sheep was also used in a few instances as indicated below. Three of the animals had been used in calorimetry experiments for many months, while the fourth was trained during the two months following operation. Individual masks (17) were used in the gasometer method, and calculations from the carbon dioxide, oxygen, and urine nitrogen were made according to Lusk (18). The two dogs which were thyroparathyroidectomized received 0.5 to 1.0 cc. doses of Parathormone (Lilly) at intervals of 24 to 48 hours, the serum calcium level being maintained between 9 and 12 mgm. per 100 cc.

RESULTS. *1. Effects of thyroparathyroidectomy: Calorigenic action.* In table 1 are shown the results of metabolism experiments on dog 6, a young, excessively active, and voracious animal. After the injections of the beef anterior lobe preparation on December 7 and 14, the basal metabolism rose 13 to 16 calories per hour, while after the operation the rise following the injections of February 7 and 28 was 6 to 8 calories. The total increase in heat production for the three days, December 15 to 17, was 663 calories, while for the three day periods, February 8 to 10, and March 1 to 3, after the operation, this increment was 367 and 285 calories respectively. Increases in rectal temperature and ventilation rate were also greater before the operation. The duration of the rise in metabolism was as great after the operation as before, the effect of the injection given on December 7 having disappeared on December 12, while that of injections given on February 7 and 28 was still marked after four days. With the less purified extract of whole glands of sheep the results were the same after the operation as before, but this preparation produced local induration at the site of injection, and a marked nitrogen loss occurred.

Dog 7 (table 2) contrasted with dog 6 in almost every respect. It was quite old, slept most of the time, and the diets which it would consume regularly contained only 33 calories per kilo or less. The nutritive index $\frac{(\text{Wt.}^{1/3}\text{gm.})}{L_{cm.}}$ was however the same as determined by Cowgill (19) for well nourished dogs, being 0.320. During play time the animal was very combative. The two injections which were given after thyroparathyroidectomy produced 837 and 646 extra calories respectively, and 6 to 7 days elapsed before the metabolism returned to normal. Details are omitted concerning three control experiments on dogs 1 and 4, which were unoperated animals of nearly the same weight as dog 7. In these the rise of the

TABLE 1

Dog 6, female

DATE	URINE N, GMS. PER DAY	BODY WT., KGM.	CALORIES PER HOUR	TOTAL R.Q.	RECTAL TEMPERATURE	VENTILATION, L. PER MIN.
12 –5–32	7.88	16.7	22.1	0.89	100.2	2.54
12– 6–32	8.08	16.7	22.5	0.88	100.1	2.52
12– 7–32	9.40	16.8	50 cc. Extract no. 095176B. (Beef)			
12– 8–32	5.78	16.6	35.4	0.76	102.6	3.73
12– 9–32	7.71	16.7	30.4	0.85	101.2	3.45
12–10–32	7.78	16.8				
12–11–32	8.64	16.9				
12–12–32	8.16	16.8	21.7	0.85	100.3	2.27
12–13–32	8.36	16.7	21.5	0.88	99.7	2.20
12–14–32	9.53	16.9	50 cc. Extract no. 095176B. (Beef)			
12–15–32	5.34	16.8	37.8	0.76	102.4	4.43
12–16–32	7.84	16.8	30.8	0.86	101.1	3.05
12–17–32			26.1	0.84	100.3	2.63
12–27–32	9.15	16.4	18.9	0.94	100.1	2.16
12–28–32	9.48	16.4	18.6	0.94	99.9	2.24
12–31–32	9.03	16.6	18.9	0.94	100.3	2.25
1– 2–33	12.18	16.6	50 cc. Extract no. 095198A. (Sheep)			
1– 3–33	10.25	16.7	33.2	0.74	103.2	10.17
1– 4–33	7.54	16.6	27.1	0.83	101.6	4.85
1– 5–33	7.82	16.7	26.1	0.86	100.8	4.73
1– 6–33	8.86	16.8	22.3	0.87	100.1	2.60
1– 7–33	9.55	16.7	20.3	0.88	100.1	2.32
1–23–33			Thyroparathyroidectomy			
2– 3–33	8.67	17.6	17.4	0.95	100.0	2.37
2– 4–33	8.60	17.7	16.4	0.93	99.4	2.00
2– 5–33	8.65	17.7				
2– 6–33	8.95	17.9	16.8	0.93	100.0	2.20
2– 7–33	9.45	17.9	50 cc. Extract no. 095176B. (Beef)			
2– 8–33	5.60	17.9	24.7	0.83	101.1	3.06
2– 9–33	5.62	18.1	21.9	0.88	100.1	2.65
2–10–33	7.07	18.3	20.3	0.90	100.4	2.53
2–11–33	8.10	18.3	20.3	0.91	99.8	2.39
2–13–33	9.30	18.1	17.9	0.96	100.0	2.30
2–16–33	9.67	18.1	17.4	0.96	100.2	2.44
2–25–33	9.48	18.1	17.7	0.94	99.7	2.42
2–27–33	8.61	18.2	16.8	0.96	100.1	2.64
2–28–33	9.93	18.3	50 cc. Extract no. 095176B. (Beef)			
3– 1–33	5.83	18.6	21.5	0.86	100.8	3.22
3– 2–33	5.34	18.7	23.3	0.85	100.8	3.20
3– 3–33	6.15	18.8	21.4	0.86	100.6	2.73

TABLE 1—*Concluded*

DATE	URINE N, GMS. PER DAY	BODY WT., KGM.	CALORIES PER HOUR	TOTAL R.Q.	RECTAL TEMPERATURE	VENTILATION, L. PER MIN.
3- 4-33	7.05	18.8	20.5	0.88	100.1	2.36
3- 5-33	7.49	18.9				
3- 6-33	8.27	18.9	20.2	0.92	99.8	2.68
3- 7-33	8.78	18.8	18.1	0.92	99.7	2.17
3- 9-33	9.30	18.7	18.4	0.91	99.7	2.21
3-10-33	10.21	18.8	18.3	0.93	99.7	2.41
3-11-33	9.48	18.9				
3-12-33	12.15	19.0	50 cc. Extract no. 095198A. (Sheep)			
3-13-33	9.90	19.1	30.0	0.81	103.2	7.02
3-14-33	7.35	19.1	27.7	0.78	101.3	5.18
3-15-33	7.65	19.1	(28.8)	0.78	101.2	5.22
3-16-33	9.53	19.0	24.0	0.78	100.1	2.90
3-17-33	10.60	18.9				
3-18-33	10.79	19.0	(25.0)	0.87	101.8	3.18

Diet: Carbohydrate 110 grams, fat 24.3 grams, protein 62.7 grams, a total of 934 calories and 10.02 grams of nitrogen per day.

Length of dog: 87 cm.

Surface area: Dec. 5, 1932, 0.732 sq.m. (Meeh-Rubner); 0.697 sq.m. (Cowgill-Drabkin); Feb. 6, 1933, 0.767 sq.m. (Meeh-Rubner); 0.715 sq.m. (Cowgill-Drabkin).

metabolism lasted 6 to 8 days, and the total calorigenic effect was 713, 773 and 1039 calories.

Nitrogen storage. Since no evident change in feces nitrogen was noted in the preceding study, and since the effect on urine nitrogen is very large, nitrogen storage was calculated from the fall in urinary nitrogen excretion, the intake being constant. Any loss of nitrogen on the experimental day was subtracted from the total deficit in urinary nitrogen occurring between the time of the injection and the return to the control level. In eight control experiments on dogs 1 and 4, in which 50 cc. doses of Antuitrin-G were given, the nitrogen storage varied from 7.55 to 16.56 grams, and averaged 11.87 grams. In four experiments on dogs 6 and 7 (tables 1 and 2) after operation, the nitrogen storage was not greater, varying from 7.78 to 10.21 grams, and averaging 9.66 grams. It should be noted however that dog 6 before the operation stored only 1.25 and 2.07 grams of nitrogen in two experiments. It is of interest that the preparation still produced nitrogen storage in dog 7 (table 2) when the calorigenic effect caused a negative energy balance. The *basal* metabolism alone, on February 17, was 564 calories per day, while the energy content of the diet, computed from actual analyses, was 561 calories. The fall in urine nitrogen is not due to retention of non-protein nitrogen, in fact the blood non-protein nitrogen actually falls (20), (12).

Water balance. Injections given to dog 7 (table 2) after thyroparathy-

roidectomy produced the usual large increases in weight, and loss of this water was definitely slower than in normal animals. In the case of dog 6 (table 1) decided increases in water intake and urine volume occurred only in two experiments in which the weight increases were also definite, though much smaller than in most animals. One of these experiments was done

TABLE 2

Dog 7, female, thyroparathyroidectomized

DATE	URINE N, GMS. PER DAY	BODY WT., KGM.	CALORIES PER HOUR	TOTAL R.Q.	RECTAL TEMPERATURE	VENTILATION L. PER MIN
1-12-33	7.48	22.5	19.0	0.79	99.9	1.93
1-13-33	7.34	22.6	19.2	0.77	100.3	2.16
1-14-33		22.6	19.3	0.81	99.8	2.00
1-15-33	7.74	22.9	50 cc. Extract no. 095176B. (Beef)			
1-16-33	3.71	23.5	28.0	0.73	101.2	2.92
1-17-33		23.6	29.5	0.71	101.9	3.19
1-18-33	5.91	23.2	24.5	0.74	100.7	2.35
1-19-33	7.10	23.1	24.5	0.76	100.9	2.64
1-20-33	7.44	23.2	22.1	0.77	99.8	2.34
1-21-33		23.2	22.5	0.73	100.3	2.61
1-22-33	8.61	23.1				
2-14-33	8.81	21.0	16.5	0.84	99.7	1.77
2-15-33	8.13	21.0	17.6	0.82	99.5	1.70
2-16-33	9.11	21.5	50 cc. Extract no. 095176B. (Beef)			
2-17-33	4.27	21.7	23.5	0.72	100.4	2.53
2-18-33	4.29	21.7	23.2	0.73	100.6	2.35
2-19-33	5.40	21.5				
2-20-33	6.84	21.5	19.9	0.74	99.8	2.13
2-21-33	7.05	21.4	19.4	0.77	99.4	2.12
2-22-33	7.52	21.4				
2-23-33	8.40	21.3	16.8	0.82		1.88
2-24-33	7.96	21.4	16.5	0.82	99.3	1.99

Diet: Jan. 12–22, Carbohydrate 82.6 grams, fat 19.6 grams, protein 55.3 grams, a total of 747 calories and 8.84 grams of nitrogen per day. Feb. 14–24, Carbohydrate 55.0 grams, fat 14.9 grams, protein 48.0 grams, a total of 561 calories and 7.68 grams of nitrogen per day.

Surface area: Jan. 14, 0.895 sq.m. (Meeh-Rubner); 0.781 sq.m. (Cowgill-Drabkin); Feb. 24, 0.863 sq.m. (Meeh-Rubner); 0.765 sq.m. (Cowgill-Drabkin).

before the operation, on December 14, and the other on February 28 after the operation. Data on daily water intake and urine volume are omitted, since weight increases indicate the essential finding,—a rise in water intake, preceding a rise in urine volume.

Autopsies. Dog 6 was autopsied September 5, 1933. The peri-aortic fat bodies (11) contained only one spherical nodule of thyroid tissue, 3 mm.

in diameter. All suspicious tissue from the neck was sectioned, and one other nodule, 5 mm. in diameter, proved to be thyroid tissue. Both this and the pericardial nodule exhibited the tall epithelium and the small amount of pale staining colloid characteristic of hyperplastic thyroid tissue. Dog 7 was autopsied March 13, 1933. No accessory thyroid tissue was found in the neck or upper mediastinum. In the peri-aortic fat bodies four small nodules were found, the smallest of which was less than 1 mm. in diameter, and the largest one 3 mm. The other two measured 1.5 × 2 × 2.5 mm., and 2 × 2 × 3 mm. The three larger nodules were identified histologically. The total amount of accessory thyroid tissue found was less than 2 per cent of that which had been removed at operation.

2. *Effect of carbohydrate supply.* In the first experiment in table 3, with the diet constant, the injection given on January 25 caused a marked fall in the basal respiratory quotient, due not only to the usual rise in fat oxidation, but to a fall in carbohydrate oxidation as well. In the second experiment 50 grams of sucrose and 50 grams of lactose were added to the diet on the day of the injection, March 26, and every day thereafter. At the peak of the calorigenic effect, March 27 and 28, all of the extra calories came from carbohydrate, fat oxidation remaining negligible. The after control period, April 5 to 21, shows that the large increase in the caloric value of the diet led to an increase in weight and a greater increase in metabolism. With the diet remaining constant and high in carbohydrate, a third injection was given on April 23. Carbohydrate oxidation remained practically unchanged, and an increase in fat oxidation again accounted for all of the extra calories.

In a fourth experiment, on dog 1, the rise in metabolism was nearly twice as great as in the first experiment on dog 4, amounting to 15.1 calories per hour or 362 calories per day. This practically equals the energy content of the added dextrimaltose (93 gm. of carbohydrate). Under these conditions the basal oxidation of carbohydrate remained unchanged; all of the extra calories came from fat. Unfortunately, panting prevented accurate calorimetry on the two succeeding days, but for the experiment on May 11 conditions were ideal. Although the metabolism was high, the respiratory rate was only 14 per minute and the ventilation 3.8 liters per minute.

Discussion. The accessory thyroid tissue which was found at autopsy does not provide an adequate explanation of the large calorigenic effects which anterior pituitary preparations produced after thyroparathyroidectomy in dogs 6 and 7. Little thyroid tissue was found in either animal, and the larger amount was found in dog 6, in which the operation had definite effects,—gain in weight beginning promptly after the operation on a constant diet (table 1), diminution of the effect of the extract on heat production, and an increase in the effect on nitrogen storage. In dog 7

TABLE 3

DATE	BASAL METABOLISM					RECTAL TEMPERATURE	BODY WT., KGM.
	Calories per hour	Total R.Q.	Carbohydrate	Fat	Protein		
Dog 4, normal female							
1933							
1-23	21.8	0.86	2.36	0.67	1.40	100.9	24.0
1-24	21.5	0.88	2.86	0.52	1.16	100.6	23.9
1-25	50 cc. Extract no. 095052A (beef) subcutaneously						24.3
1-26	27.8	0.74	0.60	2.31	0.92	100.9	24.6
1-27	29.4	0.77	1.53	2.30	0.42	100.7	24.7
1-28	28.0	0.81	2.33	1.75	0.50	100.8	24.6
1-29							24.4
1-30	24.5	0.84	2.44	1.04	1.13	100.5	24.4
1-31	23.8	0.82	1.81	1.18	1.27	100.2	24.4
2- 1							24.3
2- 2	22.9	0.86	2.69	0.76	1.13	100.5	24.3
2- 7	20.6	0.87	2.36	0.58	1.29	100.6	24.2
3-21	20.2	0.91	3.09	0.14	1.45	100.3	
3-23	21.3	0.88	2.78	0.50	1.23	100.5	23.8
3-24	19.8	0.92	3.08	0.09	1.48	100.8	23.9
3-26	50 cc. Extract no. 095204B (beef) subcutaneously 50 grams sucrose and 50 grams lactose added to diet						24.4
3-27	26.6	0.96	5.37	0.00	1.11	101.6	24.5
3-28	28.7	0.94	5.47	0.35	0.71	101.4	24.3
3-29	27.7	0.91	4.44	0.57	0.99	100.6	24.3
3-30	26.3	0.89	3.96	0.72	0.82	100.6	24.4
3-31	25.4	0.93	4.57	0.30	0.93	100.8	24.6
4- 1	27.1	0.98	5.38	0.00	1.16	100.6	24.6
4- 5	24.6	0.98	4.70	0.00	1.23	100.5	24.8
4-15	23.9	0.94	4.10	0.06	1.55	100.6	25.1
4-17	22.3	0.93	3.62	0.11	1.51	100.9	25.1
4-20	23.8	0.95	4.10	0.00	1.62	101.0	25.2
4-21	22.6	0.90	3.14	0.20	1.86	100.8	25.1
4-23	50 cc. Extract no. 095204B (beef) subcutaneously						
4-24	27.4	0.88	3.29	0.70	1.74	101.4	
4-25	28.2	0.85	2.90	0.95	1.76	101.3	
4-26	25.5	0.85	2.65	0.99	1.27	101.0	
4-27	27.2	0.89	3.75	0.68	1.29	101.1	
Dog 1, normal female							
5- 8	24.1	0.86	2.77	0.79	1.28	100.4	25.5
5- 9	23.9	0.88	3.12	0.57	1.36	100.3	25.5
5-10	50 cc. Extract no. 095176B (beef) subcutaneously 100 grams dextrimaltose no. 1 added to diet						25.8
5-11	39.1	0.80	2.90	2.55	0.82	101.5	26.1

Diets: Dog 4, same as dog 7, table 2, first diet. Dog 1, same as dog 6, table 1.
Length: Dog 4, 95 cm.; dog 1, 90.5 cm.
Surface area: Dog 4, Feb. 7, 0.936 sq.m. (Meeh-Rubner); 0.874 sq.m. (Cowgill-Drabkin). Dog 1, May 8, 0.970 sq.m. (Meeh-Rubner); 0.847 sq.m. (Cowgill-Drabkin).

the calorigenic response was practically as large as in a normal animal. Houssay and Artundo (9) suggest that the calorigenic effect after thyroidectomy may be due to direct action of a specific substance in the anterior pituitary preparation or to injection of foreign protein. The latter interpretation appears unlikely in the present experiments, since the effect on metabolism lasted 5 to 7 days after single injections which produced no induration or other local reaction, and which were followed by extensive nitrogen storage. Moreover the effect on metabolism, while present in 3 hours, is not large until later. Direct action on metabolism by a substance in the extract appears possible, since the calorigenic effect develops quite as rapidly as when thyroxin is given intravenously. In five experiments on normal dogs the peak of the effect occurred four times in the observation made 24 hours after the injection of 50 cc. of Antuitrin-G and once in the 48 hour test. An intravenous injection of 10 mgm. of thyroxin produced a calorigenic effect similar in total amount and duration, with a maximum three days after the injection. In the dog thyroxin injections of this size commonly produce their greatest effect in 45 to 70 hours (13).

The effect of the preparation on water metabolism can not, as has been suggested in the case of acid extracts (21), be readily correlated with thyroid stimulation or increase in metabolism, because a sudden increase in weight of a pound or more occurs regularly. This is due to a rise in water consumption preceding or exceeding the rise in urine volume. In three experiments in which the animals did not increase their water intake until the second day after the injection the urine volume fell, the weight increase occurring as usual. Hydration appears to be the primary effect.

Carbohydrate oxidation was obviously not interfered with by large single injections of Antuitrin-G (table 3, March 26–28). Evidence that this preparation stimulates fat metabolism directly does not appear convincing. It is true that the amount of additional carbohydrate required to prevent a rise in fat oxidation 18 hours after the last meal was much greater than the rise in metabolism, but some of the carbohydrate would probably be converted to fat during the 18 hour interval.

SUMMARY

After single injections of Antuitrin-G (beef anterior pituitary growth preparation) large calorigenic responses which could not be explained as toxic effects were observed in thyroparathyroidectomized dogs in which the amount of accessory thyroid tissue found at autopsy was small. The calorigenic effect developed at least as rapidly as that which intravenously injected thyroxin produces.

The effect of the preparation on nitrogen storage in thyroparathyroidectomized dogs was not greater than in normal dogs, although in one animal in which this effect was very small before the operation an increase

was noted after operation. The storage of nitrogen still occurred in an experiment in which the energy balance became temporarily negative as a result of the calorigenic effect.

The primary effect of this preparation on water metabolism is one of water storage; diuresis follows later. Both of these effects were still observed after thyroparathyroidectomy.

An increase in fat oxidation during the postabsorptive periods of the two days following injections occurred whether the carbohydrate intake was moderate or high. It also occurred when carbohydrate equal to the rise in metabolism was added to the diet at the time of the injection, but was completely prevented in an experiment in which the amount of carbohydrate added equalled twice the rise in metabolism.

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THEELIN CONTENT OF PREGNANCY URINE AND PLACENTA OF THE CHIMPANZEE[1]

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One of the most interesting phases of the study of internal secretions is concerned with the output of enormous amounts of female sex hormones, both ovarian and anterior-pituitary-like substances, in the urine of certain mammals during pregnancy. The interest is intensified by striking species differences which are still not satisfactorily explained. For instance, tests for pregnancy in women, which depend upon recognition of anterior-pituitary-like substance in the urine, are not applicable to certain other mammals (Snyder and Wislocki, 1931). Similar puzzling species differences have been demonstrated in ovarian hormone content of placental tissue or fetal membranes and of pregnancy urine (Allen, 1927, 1932; Hisaw and Meyer, 1929). Before generalizations are justified as to the endocrine function of the placenta or chorion and modification of balance of internal secretory function to provide for the needs of gestation, exploration of these species differences should be undertaken.

During the past year the breeding of chimpanzees in captivity at the Yale Anthropoid Experiment Station has yielded eight healthy young. Among these are a pair of twins, the first to be recorded in anthropoid apes (Yerkes, 1934). Complete records of menstrual function, mating activity, pregnancy and parturition have been compiled for these animals. With these necessary data as a background in which definitely to place a collection of samples of pregnancy urine, quantitative analyses of theelin content have been begun. Two specimens of placental tissue at term have also been extracted and tested. At present this phase of the study is only well under way, but since further breeding of these chimpanzees must be held in abeyance until this year's babies have been weaned, it has seemed desirable to publish now a brief statement of the results to date. These

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results logically accompany those reported by Zuckerman (1934) on the diagnosis of pregnancy in the chimpanzee by Aschheim-Zondek tests of urine from the same animals. Further discussion of the broader aspects of this subject with references to the literature will be found in chapters by Smith, Engle, Doisy and Allen in *Sex and Internal Secretions*, 1932, and in Zondek, 1931.

TABLE 1

Summary of theelin content of urine samples and specimens of placenta from pregnant chimpanzees

ANIMAL	SPECIMEN EXTRACTED	DATE OF COLLECTION	NUMBER OF TEST MICE USED	THEELIN CONTENT, MOUSE UNITS PER LITER OF URINE	REMARKS
No. 44 (Mimi)	66 cc. urine	11–21–33	3	None	Not pregnant; last menstruation, 10–14–33
No. 40 (Dita)	63 cc. urine	11–21–33	6	None	Last menstruation, 7–17–33
	260 cc. urine	1–15–34	11	620	Parturition, 4–21–34
	355 cc. urine	2–17–34	13	430	
	310 cc. urine	3–20–34	10	640	
	Whole placenta with cord	4–22–34	13		Placenta and cord weight 290 grams
No. 6 (Nana)	305 cc. urine	1–15–34	12	260	Last menstruation, 8–24–33
	465 cc. urine	2–17–34	8	170	Parturition, 5–2–34
	435 cc. urine	3–20–34	10	370	
	435 cc. urine	4–18–34	10	460	Placenta (part)
	Part of placenta	5– 2–34	10		Weight 100 grams
No. 38 (Fifi)	400 cc. urine	11–20–33	21	170	Last menstruation, 4–3–33 Parturition, 11–21–33
No. 56 (Lia)	Chorionic vesicle	11–13–33	7	None	Miscarriage 11–13–33

Urine samples were collected with the help of a specially designed cage, filtered to rid them of feces and debris and preserved by the addition of a few crystals of thymol. The specimens of placenta at term were recovered as soon as possible after parturition, minced, and placed in 95 per cent alcohol. Extraction was completed after arrival in New Haven.

Ten samples of sufficient amounts of urine to justify quantitative tests

for theelin have been received from four animals, three of which have previously shown positive Aschheim-Zondek reactions (Zuckerman, 1934). The urine was extracted with chloroform and the residue following evaporation was dissolved in corn oil. This process of extraction probably removes both theelin and theelol, although theelol is not very soluble in chloroform (Doisy, 1932). Extracts were injected into ovariectomized mice, the criterion of activity being the complete oestrous growth reaction of the vaginal epithelium as judged by smear preparations.

All of the urine samples from the pregnant animals have returned positive results, as indicated in the table, except one collected early in pregnancy. This was the first sample of 63 cc. volume obtained on November 21, 1933, from no. 40 (Dita). In other samples considerable quantities of theelin have been recovered. The yields range from 170 to 640 mouse units per liter. Negative results were obtained from the urine of the non-pregnant animal no. 44 (Mimi), from which 66 cc. of urine were available for extraction. At least it may be concluded that during pregnancy, large amounts of estrogenic substances are excreted in the urine.

A whole placenta with the umbilical cord attached (weighing 290 gm.) was recovered from no. 40 (Dita) soon after the birth of her baby. Part of a second placenta (weighing 100 gm.) was recovered from no. 6 (Nana). An aborted chorionic vesicle, estimated from the menstrual history to be of the second month of pregnancy, was recovered from no. 56 (Lia). Examination of this last specimen under a binocular microscope failed to reveal anything which resembled an embryo. The residues from alcoholic extraction of placental tissue and the chorionic vesicle were dissolved in corn oil and tested by the oestrous reaction in ovariectomized mice. Two hundred mouse units of hormone was recovered from each specimen of placenta. The tests of the extract of the chlorionic vesicle were negative.

We intend to extend this study of excretion of hormones in the urine and of hormone content of the placenta as additional material becomes available.

SUMMARY

Considerable amounts of estrogenic substances (theelin and theelol) have been recovered from the urine of chimpanzees at various stages during pregnancy and also from placenta of two of these animals at full term.

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THE ASCHHEIM-ZONDEK DIAGNOSIS OF PREGNANCY IN THE CHIMPANZEE

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Both the original Aschheim-Zondek pregnancy test and Friedman's rabbit test have been tried on the urine of a number of mammals other than man. Few positive results have been reported. Pregnant rats, mice, rabbits, bitches, cats, cows, swine, and the elephant are stated to react negatively (references to the original investigations are given by Engle, 1932). The urine of pregnant monkeys is also believed to be incapable of giving positive responses. Allen, Maddux and Kennedy (1931) using the Aschheim-Zondek test (repeated tests on a single animal), and Snyder and Wislocki (1931), using the Friedman test (repeated tests on two animals), obtained negative results with rhesus macaques (*Macaca mulatta*). Without revealing the basis for his belief, Zondek (1931) however states that lower monkeys ("niedere Affen") do give positive Aschheim-Zondek responses. He also reports that the urine of a pregnant orang-utan (*Pongo pygmaeus*) reacted positively, whereas that of a non-pregnant orang gave a negative result. In a personal communication, Dr. F. F. Snyder informs me that of two chimpanzees whose urine he investigated with the Friedman test, one was positive early in gestation but negative later. The second animal, tested late in gestation, gave a negative response.

Through the kind coöperation of the Director, I was able to carry out a series of Aschheim-Zondek tests on urine obtained from the chimpanzees of the Yale Anthropoid Experiment Station, Orange Park, Florida. Thirty-four tests were made between March 9, 1933 and March 21, 1934.

TECHNIQUE. Urine was collected by enticing the chimpanzee to be investigated from its living quarters into a specially constructed cage, the floor of which consisted of parallel electroplated metal rods resting upon a base which was in the form of a shallow enamelled funnel. The animal remained in the cage until sufficient urine had collected in a beaker placed beneath the spout of the funnel.

[1] The investigation was carried out during the period when the author was first a Research Associate in the Laboratories of Comparative Psychobiology, Yale University, and later a Rockefeller Fellow in the Department of Physiology and the Department of Gynecology and Obstetrics, Yale University School of Medicine.

The filtered sample of urine, into which a few thymol crystals were dropped for purposes of preservation, was sent by special delivery mail to New Haven, and it was thus possible to inject test animals within 36 to 48 hours of its collection. Through force of circumstances injections had to be delayed a few days on three occasions. According to Mazer and Goldstein (1932) well kept specimens of pregnancy urine retain their hormonal potency for at least six days, and the delays that occurred in the present investigation could therefore hardly be blamed for the negative results obtained with the particular samples concerned (dated Nov. 2, Aug. 28, and Nov. 21 in the following table). It was twice necessary to detoxicate the urine, the ether method being employed.

The tests were performed according to the routine followed in the Department of Obstetrics, Yale University School of Medicine. Rats between 21 and 29 days of age were mostly used; when rats were unavailable, mice between 17 and 23 days old were employed instead. Usually two or three test animals were injected with samples from each specimen sent. Separate controls were either kept for each specimen of urine, or group controls served for the several samples of urine that were injected on a single day. Repeated tests were sometimes carried out on the same specimen of urine. Readings were made with the help of a dissecting microscope; histological examination was not resorted to even when the result of a test was doubtful. In the chart given below, two doubtful tests are indicated by the letter "D."

RESULTS. The results of the investigation are tabulated below.

DATE OF COLLECTION OF URINE	NAME OF ANIMAL	ESTIMATED BIRTH DATE	LAST MENSTRUAL PERIOD	MATED	A–Z TEST	LATER HISTORY
Mar. 9, 1933	Pati	1920	Aug. 23, 1932	+	+	Partur.—May 14, 1933
Mar. 9, 1933	Josie	1922	Oct. 25, 1932	+	−	
Apr. 3, 1933	Josie		Oct. 25, 1932		+	
May 22, 1933	Josie		Oct. 25, 1932		+	Partur.—July 15, 1933
Apr. 3, 1933	Mona	1913	Nov. 5, 1932	+	+	Partur.—June 26, 1933
May 2, 1933	Mimi	1923	Nov. 6, 1932	+	−	Menstr.—May 10, 1933
Aug. 28, 1933	Mimi		July 2, 1933	+	−	Menstr.—Sept. 2, 1933
Nov. 21, 1933	Mimi		Oct. 14, 1933	+	−	Menstr.—Nov. 23, 1933
Mar. 21, 1934	Mimi		Mar. 20, 1934	Feb. 27, '34*	−	Menstr.—May 4, 1934
May 2, 1933	Wendy	1923	Oct. 28, 1932	+	−	
May 22, 1933	Wendy		Oct. 28, 1932	+	−	Partur.—June 27, 1933
May 22, 1933	Fifi	1918	Apr. 3, 1933	+	+	
June 5, 1933	Fifi		Apr. 3, 1933	+	+	
Nov. 20, 1933	Fifi		Apr. 3, 1933	+	D	Partur.—Nov. 21, 1933

DATE OF COLLECTION OF URINE	NAME OF ANIMAL	ESTIMATED BIRTH DATE	LAST MENSTRUAL PERIOD	MATED	A-Z TEST	LATER HISTORY
Apr. 3, 1933	Dita	1920	Mar. 2, 1933	−	−	Menstr.—Apr. 7, 1933
Aug. 28, 1933	Dita		July 17, 1933	+ from July 20	−	
Sept. 12, 1933	Dita		July 17, 1933	+	Toxic	
Nov. 2, 1933	Dita		July 17, 1933	+	D	
Nov. 21, 1933	Dita		July 17, 1933	+	+	Partur.—Apr. 21, 1934
June 5, 1933	Lia	1924	Apr. 19, 1933	+	−	
Aug. 28, 1933	Lia		Apr. 19, 1933	+	−	
Sept. 12, 1933	Lia		Apr. 19, 1933		+	
Nov. 2, 1933	Lia		Apr. 19, 1933		−	Miscarriage Nov. 13, '33
June 5, 1933	Cuba	Mar. 24, '26†		+ from May 25	−	First menstruation—July 10, 1933
Sept. 12, 1933	Cuba		Aug. 14, 1933	+	−	Menstr.—Sept. 17, 1933
Mar. 21, 1934	Cuba		Mar. 18, 1934	+	−	Menstr.—Apr. 18, 1934
May 2, 1933	May	1925		−	−	
June 5, 1933	May			+ from May 25	−	First menstruation—June 11, 1933
Nov. 21, 1933	May		Nov. 10, 1933	+	−	
Jan. 15, 1934	May		Nov. 10, 1933	Dec. 4, '33*	−	Menstr.—Jan. 18, 1934
Mar. 21, 1934	May		Mar. 8, 1934	−	−	Menstr.—Apr. 19, 1934
Nov. 2, 1933	Nana	1921	Aug. 24, 1933	+ from June 21	+	
Nov. 21, 1933	Nana		Aug. 24, 1933	+	+	Partur.—May 2, 1934
Nov. 21, 1933	Bentia‡	1926		−	−	

* Controlled mating.
† Actual birth date.
‡ Sexually immature.

Discussion. Ten of the thirty-four tests proved positive, two specimens gave equivocal answers, one sample was toxic to the test animals, and the rest returned negative results.

None of the positive results was "false."

Four of the twenty-one negatives turned out to be definitely incorrect; the later history of the animals concerned proved that they were pregnant at the time the tests were performed. It is possible that the results returned by Lia on June 5 and on August 28 were also "false negatives;" because the stage at which she miscarried on November 13 is unknown, it is however difficult to decide the question, even though the animal had not menstruated for several weeks before these particular tests were made. It is also possible that the negative returned by Dita on August 28 was fallacious; the animal gave birth 236 days later. The four definitely incorrect tests were respectively made 128 days (Josie, Mar. 9), 56 days (Wendy, May 2), 36 days (Wendy, May 22) and 11 days (Lia, Nov. 2) before the termination of pregnancy. In connection with these false

negatives, it is interesting to recall the recognized fact that the A.P.L. content of human urine diminishes from about the 5th month of pregnancy onwards, so that in man false negative A-Z tests are more frequent late in gestation than they are in its earlier phases. A similar variation in A.P.L. concentration conceivably occurs in the chimpanzee; with the exception of the first case (Josie, Mar. 9) it would help to explain the false negatives in our series, since the urines concerned were collected late in pregnancy (gestation in a chimpanzee studied by Tinklepaugh, 1933, lasted approximately 246 days, and in one reported by Yerkes, 1934, approximately 210 days).

Of the two doubtful results one (Fifi, Nov. 20) was obtained with urine collected the day before parturition; the other (Dita, Nov. 2) was given by urine collected some time during the first quarter of gestation.

The earliest positive was obtained with urine from an animal that had menstruated 49 days before, and which gave birth 183 days after the date of the specimen's collection (Fifi, May 22).

CONCLUSION

The results indicate that the Aschheim-Zondek reaction is a test of pregnancy in the chimpanzee. Because of remediable deficiencies in the technique followed in this investigation, and because of the small number of available data, it is perhaps unwise to emphasize another implication to which the results also point, viz., that as a test of pregnancy the Aschheim-Zondek reaction is less accurate for the chimpanzee than it is for man.

If reliance can be placed in the widely held belief that the urine of the pregnant rhesus macaque is unresponsive to the Aschheim-Zondek test, it is of taxonomic interest, since both the ape and man belong to the anthropoid division of the order Primates, that the urine of the pregnant chimpanzee, like the urine of man, contains A.P.L. substances.

It is a pleasure to record my appreciation of Dr. R. M. Yerkes' generosity not only in making this investigation possible, but also in supervising its progress. I owe thanks too for his permission to incorporate in the table of results supplementary data from his laboratory records. My thanks are also due to Messrs. James M. Elder, M. I. Tomilin, and Wm. C. Atwater of the Experiment Station for collecting and forwarding the samples, and to Dr. A. H. Morse and Dr. G. van Wagenen, of the Department of Obstetrics and Gynecology of Yale University School of Medicine, for providing the facilities to carry out the tests.

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STUDIES ON THE DEPRESSOR-CARDIAC REFLEX AFTER VAGOTOMY

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Kratschmer, Knoll, Brücke and others have produced slowing of the heart after vagotomy from insufflation of certain vapors into the nostril. Brücke, contrary to Cyon and Ludwig, Kazem-Beck and others, reports similar results from central stimulation of the depressor nerve. He obtained 0 to 5.5 per cent slowing of the pulse from stimulation of the depressor nerve after vagotomy and removal of the stellate and inferior cervical sympathetic ganglia.

The purpose of this paper is: 1, to repeat some of the earlier work on this depressor reflex with certain factors (known to cause slowing of the heart) controlled: 2, to determine whether the decreased pulse rate from central stimulation of the trigeminal, depressor and vagus nerves after double vagotomy suggests efferent parasympathetic impulses other than vagal, humoral effects, or an arrest of existing acceleratory impulses. These nerves were selected because the inhibited heart rate is accompanied by a rise in blood pressure from trigeminal stimulation, a drop from depressor stimulation and generally a slight rise followed by a drop from vagal stimulation.

PROCEDURE. Barbital was used for anesthesia. Blood pressure was recorded from the carotid artery or abdominal aorta. The secondary coil of a Harvard inductorium was placed at 4 to 6 cm. when stimulating the depressor nerve in the rabbit and the vagus trunk (exclusive of the sympathetic bundle) in the cat. The trigeminal nerve in rabbits was stimulated by insufflating benzol into a nostril. This stimulation was confined to the trigeminal through sectioning the olfactory tracts and inserting a cannula through the larynx into the pharynx. Since Jones has described connections between the phrenic nerve and the S. A. node and Danielopolu et al. and others before him believe that pressor and depressor fibers ascend the cervical sympathetics, these nerves were always severed.

The pulse rate per minute was calculated to within one per cent error by counting the number of pulse excursions in a 2 second interval. This was computed accurately by using finely pointed calipers under considerable magnification. In every record the rate per minute was determined

2 seconds before stimulation; between the 6th and 8th, and the 12th and 14th seconds during stimulation; and between the 14th and 16th second and 1 minute after stimulation. More readings were made if there was evidence of extraneous factors. The second, third or fourth reading always showed the maximum slowing evoked by the stimulus. The total slowing from each record was expressed in percentage and the average amount of inhibition of the pulse rate obtained from any mode of stimulation was readily obtained by adding these percentages and dividing by the number of records.

In view of the fact that previous investigators took no account of the possibility that the arrest of respiration and changes in blood pressure elicited from central stimulation of certain nerves might contribute reflexly to the total slowing of the heart, the writer will record first the results of a few preliminary experiments along this line. It was shown in a large series of experiments in which an equalizer was attached to the abdominal aorta to prevent changes in blood pressure and other experiments in which the carotid sinus reflexes were eliminated, that the usual rise or fall in blood pressure elicited from the modes of stimulation used in this study contributed so little to the total slowing of the heart that it need not be considered. A second series of experiments demonstrated that for the interval of stimulation to be used in our experiments (15 seconds) asphyxia was not a factor. However many records, before and after vagotomy, exhibited less slowing of the pulse from depressor and trigeminal nerve stimulation when artificial respiration and open pneumothorax displaced normal respiration. Consequently artificial respiration and open pneumothorax were used throughout to exclude the cardiac inhibition caused from absence of respiratory movement.

Quantitative effect of double vagotomy on these cardiac reflexes. The average amounts of inhibition of the pulse rate[1] (see under procedure for mode of computing) obtained before and after vagotomy from central stimulation of the trigeminal, depressor and vagus nerves are tabulated.

MODE OF STIMULATION	AVERAGE INHIBITION BEFORE VAGOTOMY	AVERAGE INHIBITION AFTER VAGOTOMY
Insuf. benzol, 24 rabbits	14.2% (limits 21.4 and 6.7)	9.9% (limits 17.5 and 4.4)
Depressor nerve, 16 rabbits	12.5% (limits 20 and 5.8)	8.6% (limits 12.9 and 4.5)
Vagus nerve, 10 cats	15.2% (limits 18.9 and 8)	9.7% (limits 14.7 and 6.2)

[1] In rabbits one must not mistake bigeminal pulse excursions following insufflations for inhibition. This arrhythmia is less apt to occur or takes place later if artificial respiration is used.

Occasional records showed a slower pulse after vagotomy than before. In rabbits the interval of maximum inhibition occurred between 6 and 12 seconds of stimulation. In cats it was between 12 and 14 seconds, and following double vagotomy not until several seconds after cessation of stimulation.

Decerebration. Transecting the midbrain in 4 vagotomized rabbits did not reduce the rate of the pulse from insufflation or depressor nerve stimulation by more than 2 per cent.

Effect of chordotomy at various levels. Chordotomy at the 7th C. segment (2 days to 2 weeks before) absolutely prevented all slowing of the pulse from insufflation or depressor nerve stimulation in 14 vagotomized rabbits. A few records revealed very slight pulse changes. These portrayed acceleration as often as inhibition.

On the other hand chordotomy from the 4th to the 7th T. level in 11 vagotomized rabbits generally permitted an average decrease in the heart rate of 8.1 per cent from benzol insufflation and 8.3 per cent from depressor nerve stimulation. In several instances transecting the midbrain did not reduce the inhibition by more than 1 or 2 per cent.

Removal of the first eight thoracic sympathetic ganglia. This procedure when carried out on both sides generally eliminated all pulse changes from central stimulation of the vagus in 8 vagotomized cats which had previously given the usual amount of pulse slowing from this mode of stimulation. Two of these animals ordinarily responded with 1.6 to 2 per cent inhibition and occasionally with no change or slight acceleration of the pulse.

In 5 rabbits removal of the first four thoracic sympathetic ganglia (for extent and difficulty of removal of the stellates see previous paper) obliterated all or nearly all pulse changes from insufflations.

Effect of bilateral transection of the 7th C. to the 7th T. dorsal spinal roots. This procedure was suggested by the fact that Ramón y Cajal, Lenhossék, Kúre et al., Kiss, Kahr and Sheehan (contrary to Hinsey and others) have described effector fibers in the dorsal roots; while many investigators from Sricker to Hasama (see for references) have obtained vasodilatation from stimulating the dorsal roots, and such fibers are indicated by the recent work of Bishop et al. and Rosenblueth and Cannon (1934).

The following approach was used in an attempt to prevent anemia of the spinal cord. A narrow median opening was cut through the dorsal surface of the vertebral canal with bone forceps, which was widened by a rongeur. The dorsal roots were carefully separated from the ventral by a probe and were sectioned extradurally by a small curved blade, one side at a time. In a few instances the caudal half of the dorsal roots were cut a week after the cephalic half. The arteries following the ventral surfaces of the ventral roots and the longitudinal trunks in the dura and spinal cord

were all intact and in many instances were functioning perfectly when seen through the barrel of a microscope removed from its stand.

With one vagus intact, stimulating the central end of a cut vagus in 10 cats in which the above mentioned dorsal roots had been cut for 2 to 15 days not only elicited as much but sometimes more reduction in the heart rate than was obtained when the dorsal roots were intact. After double vagotomy stimulating the vagus centrally in nine of these cats yielded an average of 8 per cent slowing of the pulse. The tenth animal (having a very slow pulse) showed no decrease in the heart rate from vagus stimulation. Eliminating this animal would bring the total average of slowing of the pulse to about the level reached for the intact dorsal root series. Figure 1 A shows the maximum cardiac inhibition (16.5 per cent) obtained from

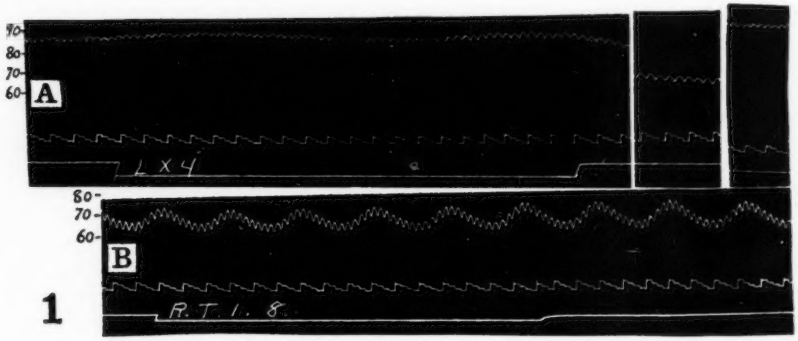

Fig. 1. Carotid tracings, time in seconds. A. During central stimulation of the left vagus of a cat in which the 7th C. to 7th T. dorsal roots were transected bilaterally; first space represents a 10 second interval and second space a 45 second interval. B. At the time of stimulating the 1st T. dorsal root.

central vagus stimulation after cutting both vagi and the above mentioned dorsal roots.

Effect of stimulating the dorsal spinal roots in the cardiac area. After cutting the 8th C. to the 2nd T. dorsal roots extradurally on one side a narrow strip of rubber was placed between the spinal cord and the cut roots to prevent spread of current to the cord. In addition the ventral roots and adjacent structures were isolated from the current by pieces of rubber about 3 mm. in diameter. These pieces of rubber had small holes at their centers through which the peripheral stumps of the dorsal roots were drawn by the aid of strings. With this protection, stimulation of these peripheral stumps (coil 7 to 10 cm.) caused no muscular contractions or any pulse changes. Figure 1 B taken during the following stimulation of the peripheral end of

the 1st T. root is typical of many records from this root, from the last C., 2nd T., and simultaneous stimulations of any two of these roots in showing an unchanged pulse following stimulation.

Effect of ergotamine. A typical result from one of ten rabbits follows in tabulated form. It should be noted for this animal that 0.55 mgm. of ergotamine tartrate[2] (Sandoz) per kilo intravenously (1:50000) produced by itself 17 per cent slowing of the pulse in one minute.

	STIMULATION BY BENZOL INSUFFLATION IN RELATION TO TIME OF INJECTION						
	1 min. before	1′ 15″ after	3′ 15″ after	5′ 15″ after	12′ 15″ after	20′ after	40′ after
Amount of pulse slowing....	18.2%	5%	0	7.2%	17%	22%	23.9%
	STIMULATION OF DEPRESSOR NERVE IN RELATION TO TIME OF INJECTION						
	2 min. before	2′ 15″ after	4′ 15″ after	6′ 15″ after	13′ 15″ after	23′ after	42′ after
Amount of pulse slowing....	9.2%	0	2.3%	10%	10%	10.8%	10.4%

The above table shows how ergotamine absolutely blocked for a few minutes all cardiac inhibition obtained from trigeminal or depressor nerve stimulation and how after 20 minutes the ordinary inhibition of the pulse rate from these two modes of stimulation was not only fully regained but increased.

The following table is compiled from several tracings taken during central vagal stimulation from a vagotomized cat in which 0.33 mgm. of ergotamine had caused 18 per cent slowing of the pulse in a minute.

	STIMULATION OF VAGUS NERVE IN RELATION TO TIME OF INJECTION							
	1 min. before	1′ 30″ after	3′ after	5′ after	8′ after	11′ after	35′ after	38′ after
Amount of pulse slowing.....	7.3%	0	0	0	0	2%	5.6%	6.4%

Effect of atropin. The following table is illustrative of many similar tests from 14 vagotomized rabbits after intravenous injection of 10 mgm. per kilo of atropin (1:1000).

[2] A dosage of 0.25 mgm. of ergotamine per kilo considerably reduced the amount of cardiac inhibition obtained from trigeminal or depressor stimulation, but it required 0.33 to 0.5 mgm. to completely block this inhibition. It should be noted for these experiments in which artificial respiration was used that the larger doses of ergotamine eliminated all premature systoles which may follow insufflation.

	STIMULATION BY BENZOL INSUFFLATION IN RELATION TO TIME OF INJECTION						
	1 min. before	1′ 15″ after	3′ 15″ after	5′ after	10′ after	30′ after	60′ after
Amount of pulse slowing....	15%	5.9%	7%	6%	7.5%	11%	18%
	STIMULATION OF DEPRESSOR NERVE IN RELATION TO TIME OF INJECTION						
	2 min. before	2′ 15″ after	4′ 15″ after	6′ after	11′ after	33′ after	64′ after
Amount of pulse slowing....	7%	0	0	0	4%	5.4%	10.1%

It is apparent from this table that there is an interval of several minutes in which atropin greatly diminishes or obliterates the cardiac inhibition obtained in vagotomized rabbits from trigeminal and depressor nerve stimulation.

Effect of physostigmine. After intravenous injection of very large doses (1 mgm. per kilo) of physostigmine so much slowing of the pulse (40 per cent or more) resulted from the drug that no further inhibition could be obtained from trigeminal or depressor nerve stimulations for a long time. When given in doses of 0.25 mgm. per kilo the results were variable. Occasionally when these previous depressor reflexes yielded considerable slowing of the pulse, physostigmine for a time reduced or obliterated all slowing of the pulse from these reflexes; and again when these previous inhibitions of the pulse rate were slight, physostigmine for a time increased the cardiac inhibition from these reflexes.

Discussion. The fact that such pronounced cardiac inhibitory reflexes were possible in the absence of the vagus was an unexpected result that should be emphasized.

Brücke's conjecture that stimulation of depressor nerve fibers causes an inhibition of the tonus of the acceleratory nerves has been considerably strengthened by Bronk et al., demonstrating that raising the blood pressure in the carotid sinus arrested for a short time sympathetic currents that were previously present. The writer's results (which in part are only confirmatory) lend the following support to this hypothesis: 1. After vagotomy the integrity of the spinal cord to the 4th T. segment and of the upper thoracic sympathetic ganglia is essential for slowing of the pulse to take place from central stimulation of the trigeminal or vagus nerves. 2. Ergotamine obliterates for a short time the above mentioned depressor reflexes on the heart rate after vagotomy. 3. These depressor reflexes are not usually effective after vagotomy if the pulse is very slow or has been greatly inhibited by physostigmine. 4. These depressor reflexes persist after eliminating all possible routes for parasympathetics except the

ventral roots. 5. That acceleratory impulses are constantly going to the heart by way of the sympathetics is indicated by the pronounced slowing of the pulse which occurs in vagotomized animals immediately after stellectomy.

After it was shown that stimulating certain depressor nerves still elicited considerable slowing of the heart following transection of the vagi, cervical sympathetics, phrenics and the dorsal roots of the cardiac region of the cord, the only possible route for parasympathetic impulses to reach the heart would be through the ventral roots. Parasympathetic fibers however are not included in our present anatomical set up for the thoracic ventral roots and they would be very difficult to demonstrate without an oscillograph. But if these fibers are present in the thoracic ventral roots, they could contribute to or even account for the slowing of the pulse in the above mentioned conditions, and much of the evidence used in support of the previous hypothesis would also favor this supposition.

It would appear that transportation of acetylcholine to the heart from a distant source is not an important factor in this problem for both Bain and Feldberg have made it clear that the acetylcholine liberated from stimulation of the lingual nerve is instantly destroyed by blood.

If all preganglionic autonomic fibers liberate acetylcholine upon stimulation as indicated by Feldberg and Gaddum's recent work on the cervical sympathetics, and if sufficient acetylcholine is formed at a synapse from stimulation of a preganglionic fiber to evoke an effector impulse in the cell as suggested by these authors, a volley of sympathetic impulses might produce sufficient acetylcholine at the cardiac synapses to temporarily block all acceleratory impulses.

Rothyln, Heymans et al., Wright, Allen, Rosenblueth and B. Cannon have demonstrated that certain depressor reflexes on blood pressure or on the heart rate are diminished by ergotamine before or after vagotomy. But so far as known to the writer these cardiac reflexes have not been completely arrested by ergotamine after vagotomy as was shown in this study.

If depressor nerve stimulation arrests acceleratory impulses, this inhibition probably takes place in the reticular formation of the brain stem[3] for the writer (1927) demonstrated that no fibers from the solitary tract nucleus (terminal nucleus for all vagal depressor fibers) descend the spinal cord, but synapse presumably in the formatio reticularis.

[3] The fact that after vagotomy the same stimulation of the trigeminal nerve produces depressor effects on the heart simultaneously with pressor effects on blood pressure is an additional point in favor of separate effector areas in the formatio reticularis for the heart and for blood pressure.

SUMMARY AND CONCLUSIONS

Under the conditions of these experiments the average inhibition of the pulse rate obtained from central stimulation of the trigeminal, depressor and vagus (cats) nerves was 14.2, 12.5 and 15.2 per cent before, and 9.9, 8.6 and 9.7 per cent after vagotomy.

After vagotomy the slowing of the pulse obtained from central stimulation of the trigeminal and depressor nerves (rabbits) and the vagus (cats) was generally obliterated by transecting the spinal cord at the 7th C. or removal of the first six thoracic sympathetic ganglia. It was not to any significant extent affected by midbrain transection, sectioning of the spinal cord at the 7th, T. or cutting the 7th C. to the 7th T. dorsal roots. This inhibition of the heart rate was arrested for a short time by ergotamine and greatly reduced or obliterated by atropin and very large doses of physostigmine.

Faradization of the 8th C., 1st or 2nd T. dorsal roots did not alter the pulse rate.

This study indicates that temporary suppression of cardiac acceleratory impulses from an effector area below the midbrain is the chief cause after vagotomy of the slowed pulse obtained from depressor nerve stimulation. Other possible factors are noted in the discussion.

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BLOOD PICTURE, REPRODUCTION, AND GENERAL CONDITION DURING DAILY EXPOSURE TO ILLUMINATING GAS

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The greatest ever-present source of danger from chronic carbon monoxide poisoning is illuminating gas (1). Very many individuals breathe gas-contaminated air, it being a hazard to which anyone may be exposed.

The following report is based upon a study of chronic illuminating gas poisoning induced in *Mus norvegicus albinus*, the Ames-Wistar strain.

A closed circuit respiration apparatus (fig. 1) was devised by means of which rats could be exposed to varying percentages of gas-air mixture and be maintained in comfort for hourly intervals. The apparatus enclosed 20,700 cc. of air. By trial and error it was found that addition of 300 cc. of illuminating gas to this volume of air made a mixture just sublethal for adapted rats placed therein for one hour. In this interval the hemoglobin became 60 to 70 per cent saturated with carbon monoxide. Adaptation was brought about by gradually lengthening the daily exposure period from an initial ten minutes on the first day to one hour in the course of about three weeks.

The percentage composition of the gas used was: CO 23.5; CH_4 12.4; N_2 19.37; H_2 27.9; CO_2 6.9; O_2 1.14; illuminants 8.8. The percentage of illuminating gas breathed by the rats was 1.43 and of carbon monoxide 0.34. The major influences of the gas are attributed to its carbon monoxide content.

BLOOD STUDIES. *CO saturation.* The saturation of the hemoglobin with carbon monoxide was measured by the method of Sayers and Yant (7). The percentage saturation for different intervals from one minute to two hours after one hour exposure are presented in figure 2. The saturation at death varied from 75 to 85 per cent, which is the usual lethal saturation for pure carbon monoxide. The control rats showed 0 to 5 per cent carbon monoxide hemoglobin. Gassed rats did not become free of carbon monoxide in 24 hours, the readings varying between 0 and 10 per cent. No measure of the other constituents of the gas in the blood was attempted.

Fragility tests. Resistance of the red blood corpuscles to hypotonic salt

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

solutions showed no change until after the gassing had been continued for about 200 days. In small groups of animals exposed for more than 200 successive days slightly increased fragility was exhibited by all gassed rats over non-gassed litter mates.

Cell plasma ratios. By the hematocrit method of Van Allen (8) the average per cent of corpuscles for controls was 58.8 as compared to 75 for rats which had been gassed for about 200 successive days. Varying increases of less magnitude were observed in accordance with the duration of the experimental period, sex, and age of the animals compared. The cell percentage correlated directly with the hemoglobin percentage.

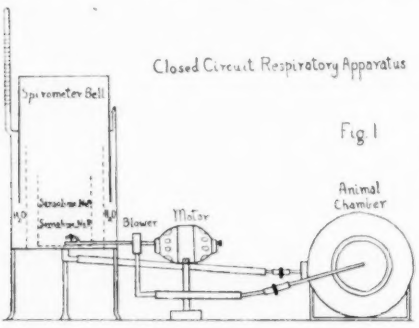

Fig. 1

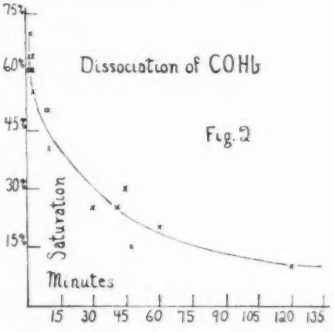

Fig. 2

TABLE 1

AGE OF RATS IN DAYS	NUMBER OF DAYS EXPOSED TO GAS	HB IN GRAMS GASSED RATS	HB IN GRAMS CONTROL RATS	MEAN DIFFERENCE
150 ♂ and ♀	125	16.62 ±0.43	13.96 ±0.07	2.66 ±0.48
199 ♀	50	17.97 ±0.44	13.04 ±0.47	3.9 ±0.64
255 ♀	119	19.8 ±0.3	15.2 ±0.13	4.6 ±0.33
309 ♀	178	17.68 ±0.55	14.56 ±0.53	3.1 ±0.26
351 ♀	219	15.84 ±0.9	13.68 ±0.17	2.2 ±0.5
394 ♀	262	17.02 ±0.23	13.33 ±0.33	3.7 ±0.4

Hemoglobin. Hemoglobin determinations were made at weekly or biweekly intervals on control and experimental animals. The improved Newcomer (4) method was used throughout; all readings are expressed in grams per 100 cc. Table 1 presents typical readings. The mean differences are highly significant; high hemoglobin percentage was maintained throughout 262 days of treatment. Animals of like age, sex and gassing history only are comparable. The highest reading obtained among female rats was 25.5 grams and among males 21.0 grams.

During the war it was found that either anemia or polycythemia may be produced in man by chronic carbon monoxide poisoning (2). Sayers (6) reports polycythemia in man.

REPRODUCTION. Early in the course of the experiments it was observed that rats which became pregnant gave rise to inferior offspring, which usually died. After more prolonged treatment dead feti were born and later on no pregnancies ensued.

In order to study this situation mating histories were collected for gassed males when mated with normal females and for gassed females mated with normal males.

Mating histories—gassed males. Six male rats which had been gassed for two weeks were caged continuously with two normal females each, for about thirty days. Two pregnancies ensued but no young survived.

Six male rats caged separately and gassed for fifty successive days were placed for four-day intervals with normal females upon three separate occasions. One pregnancy ensued but no young survived.

Three male rats which had been gassed for seventy-five successive days were mated with two normal females each upon two separate occasions. No pregnancies ensued.

Other isolated matings after more prolonged treatment of the male parent resulted negatively. Weight records of the females above listed gave no evidence of resorptions.

Nine ungassed male litter mates of the above listed rats living in the same room, eating the same diet, receiving the same care and similarly mated gave rise to fourteen litters during the same experimental period. Eleven of these litters grew to maturity.

At autopsy the testis and epididymis of the above rats were examined for spermatozoa by the hanging drop method. Early in the treatment sluggishly motile sperm were the rule; non-motile sperm were found after 60 days' treatment whereas later no sperm cells could be seen in the preparations. The litter mate controls exhibited actively motile spermatozoa in all cases. Histological examination of the testis and epididymis from rats gassed 120 days showed sperm cells to be present. Testes of rats gassed for 120 successive days weighed from one-half to one-third those of their litter mate controls.

Mating histories—gassed females. Seven female rats, gassed for a period of three months, were caged continuously with normal males of positive mating history. Thirteen pregnancies ensued. This is half the number that occurred among their litter mate controls for the same interval. The number of young per litter was less than average, but the exact number is unknown since the young were usually destroyed by the mother at birth or soon after. From the young born only two survived to weaning age.

Among five female rats gassed for 150 successive days no pregnancies could be induced, whereas the litter mates bore normal young when mated with the same male and during the same period.

Among three females gassed for two hundred fifty successive days no pregnancies could be induced, whereas the ungassed litter mate bore young when mated on the same day with the same male.

The oestrous cycle in a group of 12 normal rats studied showed a mean length of 4.74 $\pm$0.144 days over an observation period of 73 days. The mean cycle length in these rats became 5.56 $\pm$0.2 days during a subsequent gassing period of 68 days. The mean difference is 0.82 $\pm$0.24 day, which is a significant difference. The cycle length did not seem to be further prolonged in rats gassed 250 days. The mean cycle length for all control rats studied was 4.7 $\pm$0.1 days.

Histological study of the ovaries of gassed rats showed Graäfian follicles and corpora lutea of normal appearance. The size of the ovaries seemed to be somewhat reduced. Great excess of red blood corpuscles was apparent in histologic sections of all tissues.

Wells (9) using pure carbon monoxide as a source of chronic poisoning in rats reports a high percentage of abnormal prenatal development and

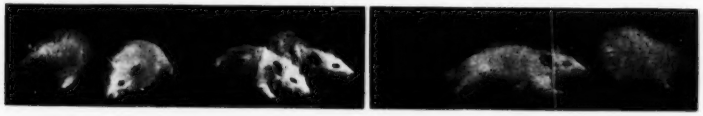

Fig. 3 Fig. 4

Fig. 3. Female rats (litter mates). Left—ungassed controls. Right—Exposed to 1.43 per cent illuminating gas-air mixture one hour daily for 154 successive days.

Fig. 4. Male rats (litter mates). Left—ungassed control. Right—Exposed to 1.43 per cent illuminating gas-air mixture for 150 successive days.

small and abnormal litters. Rossiter (5) reports that men, continually exposed to air contaminated with carbon monoxide, complain of loss of sexual desire; McCombs (3) states that men surviving acute carbon monoxide poisoning are impotent. Williams (10) states that poisoning with illuminating gas may lead to death of the human fetus and its subsequent expulsion from the uterus.

General condition. Daily exposure to gas proved deleterious to the well being of the animals (figs. 3 and 4). Body weight was consistently lower in gassed rats. Decreased appetite was apparent. Muscle tone and tissue turgor were reduced. The hair became unkempt and a typical alopecia ensued, old hair being retained and new hair scanty (figs. 3 and 4). No predisposition to infection was incurred. The lungs of gassed rats appeared normal at autopsy except for occasional emphysema and excess of red blood cells. The deleterious influences were more serious in the male. The differences in weights between control and experimental animals were greater among males; the hemoglobin response was less; and

the procreative power was more greatly reduced, this occurring earlier in the experimental period. The number of male rats which died from acute poisoning during the adaptation period was always greater than the number of female rats that died during this time.

SUMMARY AND CONCLUSIONS

A condition of chronic carbon monoxide poisoning was induced in albino rats by exposure for one hour daily to a sublethal percentage of illuminating gas. The most prolonged experiment was continued for two hundred sixty-four days.

The hemoglobin percentage became significantly increased and remained high throughout the course of all experiments. The cell plasma ratio ranged from 58.8 in ungassed to 75 in gassed rats. Gradations between these figures were found in accordance with the duration of the experimental period. The percentage of red cells correlated directly with the percentage of hemoglobin.

Slightly increased fragility of red blood corpuscles was induced by prolonged treatment. Both male and female rats became sterile after prolonged treatment. The body weight of gassed rats was consistently lower than that of their litter mate controls. The well being of the animals was below standard but no respiratory or other infection occurred among them.

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THE EFFECT OF INCREASED AIR PRESSURE ON VITAL CAPACITY, EXPIRATORY FORCE AND BREATH-HOLDING ABILITY

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Physiological tests to determine physical fitness have long been used on military groups, particularly aviators. The determination of vital capacity, expiratory force and breath-holding ability is considered of value in estimating physical fitness. Thus in the study of the physical changes in the human body incident to increased air pressure it was thought advisable to determine the vital capacity, expiratory force and the ability to hold the breath under this condition.

METHOD. Twenty-five subjects who were experienced divers and accustomed to taking pressure almost daily acted as the subjects for this study.

The subjects were sufficiently trained in the three tests at atmospheric pressure and after this period of training the tests were conducted under increased air pressure. Four determinations were then made on each subject in two separate exposures at a pressure of 75 pounds gauge or six atmospheres absolute. It is thus apparent that the data collected show the real increase of the vital capacity, expiratory force and the ability to hold the breath caused by the increased pressure of six atmospheres.

Vital capacity was determined by the use of a standard type balanced spirometer. The subjects while standing were instructed to inspire as deeply as possible and then to expire as much as possible into the spirometer.

Expiratory force was measured by the use of a mercury manometer of the type used for determining blood pressure, to which was attached by pressure tubing a mouthpiece similar to that used in the mine rescue apparatus. The subjects while seated were required to blow steadily and force the column of mercury as high as possible.

In breath-holding the subjects reclined on a cot and were instructed to expire once as deeply as possible, then inspire fully and hold the breath as long as possible. During this time the subjects were carefully watched for signs of cyanosis and were asked why they had to give up.

RESULTS. *Atmospheric pressure.* The findings for the group are as follows:

	Average	Standard deviation
Age, in years	30.84	4.36
Height, in inches	69.44	2.27
Weight, in pounds	161.20	18.75
Vital capacity, in liters	4.756	0.579
Expiratory force, in mm. Hg	172.60	35.25
Breath-hold, in seconds	91.00	27.45

A more detailed presentation of the findings at atmospheric pressure may be had by reference to table 1 where the complete data for the group are given. It will be noted that the greatest vital capacity was 6.21 and that the least was 3.61 liters. The greatest expiratory force at atmospheric pressure was 240 mm. Hg while the least was 90 mm. Hg. The longest time the breath was held was 2 minutes and 47 seconds while the shortest was 52 seconds.

It is apparent that the group is above normal and coöperated to the fullest extent, for the average of vital capacity, expiratory force and breath-holding is high when compared to the findings of others.

Thus Schneider (1921) investigating the physical efficiency of 128 aviators found the average vital capacity in liters was 4.33 and the average expiratory force in millimeters mercury was 123.13; while Cripps (1924) reporting on 950 men of the Royal Air Force found the average vital capacity in cubic centimeters was 4,604.8 and the average expiratory force in millimeters mercury was 133.13; and Shilling (1933) examining 420 officers and men of the U. S. Navy submarine service found the average vital capacity in liters was 4.519 and the average expiratory force in millimeters mercury was 114.2. Schneider (1930) reported that the mean breath-holding ability of 318 American aviators in France was 67.8 seconds.

Pressure of six atmospheres. Under the influence of this pressure the three tests gave the following averages:

	Average	Standard deviation
Vital capacity, in liters	5.064	0.657
Expiratory force, in mm. Hg	199.20	40.02
Breath-hold, in seconds	216.50	117.52

The complete data for the group are given in table 1.

Vital capacity was found to increase from an average of 4.756 liters at atmospheric pressure to 5.064 liters under pressure which is an average increase for the group of 308 cc. While this is not great it will be seen by reference to table 1 to be significant, for every subject except no. 8 showed at least some increase under pressure which amounted in one case to as much as 22.6 per cent over the atmospheric normal for this subject. The actual increase for this subject was 930 cc.

Expiratory force showed more variation, 22 subjects showing an increase

under pressure, 2 showing a slight decrease and 1 showing no change; yet the average of the group increased from 172.6 mm. Hg at atmospheric pressures to 199.2 mm. Hg at six atmospheres. Subject 13 had the greatest expiratory force with 240 mm. Hg at atmospheric pressure and 300 mm. Hg at six atmospheres, but subject 8 showed the greatest percentage increase with actual figures of 154 mm. Hg at atmospheric pressure and 232 mm. Hg at six atmospheres.

TABLE 1

SUBJECT NUMBER	ONE ATMOSPHERE			SIX ATMOSPHERES			PERCENTAGE INCREASE		
	Vital capacity	Expiratory force	Breath hold	Vital capacity	Expiratory force	Breath hold	Vital capacity	Expiratory force	Breath hold
1	4.12	230	74	5.05	260	226	22.6	13.1	205.4
2	4.72	155	76	5.13	190	150	8.6	22.6	97.4
3	4.21	142	103	4.28	158	198	1.6	11.3	92.2
4	5.36	170	106	5.62	220	270	4.9	29.4	154.7
5	4.28	142	56	4.42	165	88	3.3	16.2	57.2
6	5.46	190	91	6.00	204	269	9.8	7.4	195.6
7	4.42	118	52	4.78	150	116	8.1	27.1	120.5
8	4.30	154	92	4.29	232	123	−0.2	50.6	33.6
9	3.82	190	70	4.04	248	189	5.7	30.5	170.0
10	4.38	196	106	4.72	228	255	7.7	16.3	140.5
11	4.56	214	167	4.84	260	432	6.1	21.4	158.6
12	5.42	162	93	5.81	182	189	7.1	12.3	103.2
13	4.88	240	77	5.20	300	124	6.5	25.0	61.0
14	6.21	178	133	6.52	210	617	5.0	18.0	363.9
15	4.08	188	55	4.36	174	76	6.9	−8.0	38.1
16	5.19	124	109	5.39	168	363	3.9	35.5	133.0
17	5.00	152	70	5.24	152	92	4.8	0.0	31.4
18	5.20	190	128	5.22	210	220	0.4	10.5	71.8
19	5.08	200	55	5.33	190	202	5.0	−5.0	267.2
20	5.34	235	97	5.90	238	300	10.5	1.3	209.2
21	4.96	180	66	5.44	210	150	9.7	16.7	127.2
22	4.90	200	97	5.00	214	258	2.0	7.0	165.9
23	4.74	145	100	5.31	170	222	12.1	17.2	122.0
24	4.55	90	80	4.74	108	135	4.1	20.0	68.7
25	3.61	160	117	3.68	180	185	1.9	12.5	58.1

Breath-holding showed a great increase at a pressure of six atmospheres. By reference to table 1 it will be noted that 16 subjects showed an increase of over 100 per cent. The greatest increase for the entire group was 363.9 per cent and the lowest was 31.4 per cent. The subject showing the greatest percentage increase held his breath at atmospheric pressure 2 minutes and 13 seconds, while at six atmospheres pressure he held his breath for 10 minutes and 17 seconds. The distribution of the subjects as to breath-holding ability is as follows:

10 to 11 minutes	1
7 to 8 minutes	1
6 to 7 minutes	1
5 to 6 minutes	1
4 to 5 minutes	4
3 to 4 minutes	7
2 to 3 minutes	6
1 to 2 minutes	4

The average time for the group under pressure was 216.5 seconds, while under atmospheric conditions the average time of the breath-hold was only 91 seconds which is an average increase for the group of 125.5 seconds.

SUMMARY

Data have been presented showing the normal findings for the vital capacity, expiratory force, and breath-holding of a group of trained divers at atmospheric pressure and under a pressure of six atmospheres absolute.

Vital capacity showed a moderate increase in all but one subject under pressure.

Expiratory force increased in 22, remained the same in 1, and decreased in 2 subjects under pressure.

The ability to hold breath showed a great increase in every subject under pressure.

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THE EFFECT OF THYROIDECTOMY AND THYROXINE ON THE RESPONSE OF THE DENERVATED HEART TO INJECTED AND SECRETED ADRENINE[1]

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The effect of the thyroid on the response to adrenine of structures innervated by the sympathetic system has been widely studied, but with varying results. The literature on the subject is concerned with two main groups of experiments; those in which a thyroid extract or thyroxine was administered over a long period of time, and those in which the tests were immediate. In view of what is now known regarding the delayed action of the thyroid hormone (Kunde, 1927), it is unlikely that any results from acute experiments are specific. A possible explanation of positive experiments which have been reported is that the thyroid preparations which were used were alkaline or contained protein, since both conditions are known to increase the response to adrenine (Feldberg and Schilf, 1927; Flatow, 1928). Prolonged administration of thyroid preparations, in most cases, causes an increase in the response to adrenine. Thus, after thyroid feeding, Eppinger, Falta and Rudinger (1908) obtained an adrenine mydriasis in normal dogs. Bergwall and Kuchinsky (1931) observed an increase in the sensitivity of the iris to adrenine after thyroxine. An augmented response of the blood pressure and isolated heart to adrenine is also produced after prolonged thyroid and thyroxine administration, according to Santesson (1919) and Lütolf (1930). König (1928), on the other hand, was unable to obtain an increase of the pressor reaction.

Observations on the effect of lack of thyroid secretion on the sensitivity of structures to adrenine are inconclusive. Total thyroidectomy, according to Eppinger, Falta and Rudinger (1908), greatly diminishes and sometimes abolishes adrenine glycosuria in dogs. Pick and Pineles (1908) were unable to confirm this finding on mature goats and rabbits. Bornstein (1930) noted the same response of perfused limb vessels to adrenine in normal dogs and in those which had been thyroidectomized for some weeks. On the other hand, Schermann (1927) found a marked decrease in the reaction of

[1] This work was aided in part by a grant from the Proctor Fund of the Harvard Medical School.

the isolated heart to adrenine in thyroidectomized rabbits. Similar results on the blood pressure of rabbits have been obtained by König (1928). Schliephake (1928), in studying the pressor response to adrenine before and after thyroidectomy, obtained in two dogs practically no change in response, but in a third a considerable diminution.

Certain objections may be raised to most of the previous experiments. In no case was the effect of thyroid administration or removal checked by records of oxygen consumption. Nearly all experiments were complicated by anesthetics, and many by the use of an isolated organ as a test object. Furthermore, with one or two exceptions control experiments were not made on the same animal, so that no allowance was made for possible variation of the individual responses to adrenine. It seemed highly desirable, therefore, to test quantitatively the effect of thyroidectomy and thyroxine on the response to standard doses of adrenine, with use of a specially prepared animal which could be observed over a long period of time and in which a number of control experiments could be made at different stages. For this purpose the denervated heart was selected as an indicator of the sensitivity to adrenine, and its response was followed after total thyroidectomy and after thyroxine administration. Observations of the animal's basal metabolic rate were made throughout the course of the experiment.

Observations on injected adrenine. *Methods.* Cats were used for all experiments. Under ether anesthesia the right adrenal gland was removed, the left demedullated and completely denervated by section of the major and minor splanchnics and removal of the upper portion of the left abdominal sympathetic chain. The hepatic nerves also were severed. At a second operation the heart was isolated from the central nervous system, according to the method described by Cannon, Lewis and Britton (1926).

About two weeks after the denervation, when the heart is known to be fully sensitized, experiments were begun. The animals were trained to lie quietly on one side on an animal board, with one hind leg held in extension. The heart rate was found by means of a stethoscope strapped to the cat's chest and was counted always by the same investigator. At the beginning of the experiment a needle was inserted into the saphenous vein and strapped in place. The needle was kept from filling with blood by previously covering its large end with a cap-like device made from a piece of rubber tubing about 1 cm. in length, closed at one end with rubber cement. Before being inserted into the vein the needle and its rubber cap (having a capacity of about 0.2 cc.) were filled with normal salt solution. Intravenous injections could be made into the rubber cap whenever desired without in any way disturbing the animal. Injections of adrenine were always followed by 0.5 cc. of salt solution. The injection time for the adrenine

was usually three seconds, and this was kept as constant as possible. A dilute solution was made up fresh for each experiment from a 1:1,000 solution of adrenalin (Parke, Davis), and precautions were taken to avoid its exposure to heat or sunlight.

With the animal at rest on the board the heart rate was counted and recorded every 15 seconds until a constant level was reached. The adrenine injection was then made and the heart rate noted immediately thereafter. The counting was continued for two minutes. All experiments were done on animals which had previously fasted for 18 to 24 hours.

The thyroid gland was removed according to the method described by Dott (1923). An effort was made to identify and spare the parathyroids, and this was possible in all but a few cases. After thyroidectomy the response to adrenine was closely followed. In four animals injections were made 2, 4, 6 and 10 days after the operation, while in two others they were made 1, 2, 4, 7, 11, 14, 18 and 24 days after removal of the gland.

A Benedict portable respiration apparatus was used to determine the oxygen consumption of the cats. It had an outside electric blower and was attached to a water-sealed chamber. Soda lime was used for CO_2 absorption. Observations made by this method on our cats, while normal and fasting, were of the same order of magnitude as those reported by Aub, Bright and Forman (1922).

Response of the denervated heart to standard solutions of adrenine. It was necessary to determine whether the response of the denervated heart to standard doses of adrenine remained constant. Control injections were made, therefore, on the seven animals studied on three to six different days, and in some cases duplicate injections were made on the same day. The standard solutions employed were 0.5 cc. of 1:500,000 and 0.5 cc. of 1:5,000,000. In three cats the two solutions were used; in three others only the 1:500,000 solution; and in one, only the 1:5,000,000. As a rule the animals were not disturbed by the injection and remained quiet throughout the experiment.

During the first quarter-minute after the administration of adrenine, acceleration of the heart began; it reached a maximum during the second quarter and then the rate began to decrease. Throughout our records the difference between a control quarter-minute period before, and the second quarter-minute after the injection is considered as the maximal response. Since the basal heart rate varied from animal to animal, the increments have been calculated as percentage rises over the basal so as to be made comparable. The rate response of the heart of an individual animal to a given dose of adrenine was found to be remarkably constant, both on the same day and on different days. In one experiment five injections of 1:500,000 solution of adrenine over a period of three days gave a maximum rise of either 15 or 16 beats (table 1). Similar results were obtained on the other six cats.

The effect of thyroidectomy. After thyroidectomy a lowering of basal heart rate was observed in all the animals except one (table 2). The maximal decrease was not observed for some days and usually amounted to 15 to 20 beats per minute. Schermann (1927) has reported a similar result in thyroidectomized rabbits.

TABLE 1

Response of the denervated heart to intravenous injection of 0.5 cc. of 1:500,000 solution of adrenine. Cat 79

DATE	TIME AFTER THYROIDECTOMY	HEART RATE EVERY 15 SECONDS				OXYGEN CONSUMPTION CALORIES PER KGM. PER HOUR		WEIGHT
		Basal before injection	Maximal after injection	Increase	Percentage increase over basal	Control	Percentage decrease	
	days							*kgm.*
April 15		26	42	16				
April 17		25	40	15				
		26	42	16				
April 20		26	42	16				
		26	41	15	60.5	2.22		3.60
April 23		Thyroid completely removed						
April 24	1	24	39	15				
		25	38	13	57.1	2.04	7.8	3.64
April 25	2	23	36	13				
		24	36	12	53.2	2.00	9.6	3.52
April 27	4	23	35	12				
		23	34	11	50.0	1.94	12.3	3.54
April 30	7	21	29	8				
		21	30	9	40.5	1.24	43.9	3.68
May 4	11	20	27	7				
		21	28	7	33.8			
		21	28	7				
May 7	14	22	31	9				
		20	30	10	46.2	1.13	48.9	3.84
		21	31	10				
May 11	18	21	32	11				
		21	31	10	50.0	1.62	37.4	3.74
May 17	24	20	31	11				
		20	30	10	52.5	1.71	33.2	3.74

In all experiments, after removal of the thyroid, the response of the heart rate to a standard dose of adrenine decreased (table 2). The protocol of a complete experiment in one animal is shown in table 1. The decrease began in three cats as early as one day after thyroidectomy, before any noteworthy change in oxygen consumption took place (fig. 1A and B). On

the second day a slight but definite diminution in metabolism usually occurred, and also an unmistakable lessening in the response of the heart to injected adrenine. Thereafter the rate response and the oxygen consumption continued to decrease for a variable number of days. The time at which the maximal decrease in the heart rate was observed was different in the seven animals, the shortest period being 2 days and the longest 11 (table 2).

The amount of rate decrease manifested considerable variation. The greatest effect observed, with 1:500,000 dilution, was a diminution of 58 per cent, the smallest a diminution of 30 per cent, with an average of 44 per

TABLE 2

The maximal change in the response of the denervated heart to adrenine after thyroidectomy

CAT NUMBER	BEFORE THYROIDECTOMY			AFTER THYROIDECTOMY			PERCENTAGE DECREASE IN RESPONSE*	DAYS AFTER OPERATION	PERCENTAGE CHANGE IN B.M.R.
	Average basal 15 sec.	Average increase 15 sec.	Per cent increase over basal	Basal 15 sec.	Minimal increase 15 sec.	Per cent increase over basal			
1:500,000 solution of adrenine									
57	36.2	10.7	29.6	32.0	7.0	21.0	39.1	9	−31.8
67	31.0	12.5	40.0	26.0	5.0	19.2	52.0	6	−20.9
79	25.8	15.6	60.5	20.7	7.0	33.5	44.6	11	−44.0
80	30.7	15.0	48.8	29.0	8.0	27.6	43.5	6	−32.4
81	29.8	14.7	47.7	28.5	9.5	33.3	30.2	2	−10.3
86	29.8	14.4	48.3	29.5	6.0	20.3	58.0	4	−21.2
1:5,000,000 solution of adrenine									
57	36.2	5.0	13.9	32.0	3.0	9.2	33.9	9	−31.8
59	31.0	6.5	20.6	26.0	2.0	7.6	38.0	6	−28.4
80†	30.7	5.0	16.2	30.0	1.0	3.3	79.7	6	−32.4

* Control response taken as 100.

† Second injection on sixth day.

cent for six experiments. Similar changes were noted when the dilution was 1:5,000,000 (table 2).

After thyroidectomy there is a qualitative relationship between the response to adrenine and the metabolism, in that a decrease takes place in both at approximately the same time. In different animals, however, the drop in the rate response is not proportional to the drop in oxygen consumption (table 2). Furthermore, the maximal decrease in the rate response usually precedes the maximal decrease in metabolism by two or three days.

A spontaneous rise in metabolism was observed in four of our animals. In one cat it reached its pre-operative level in 9 days, in another it returned

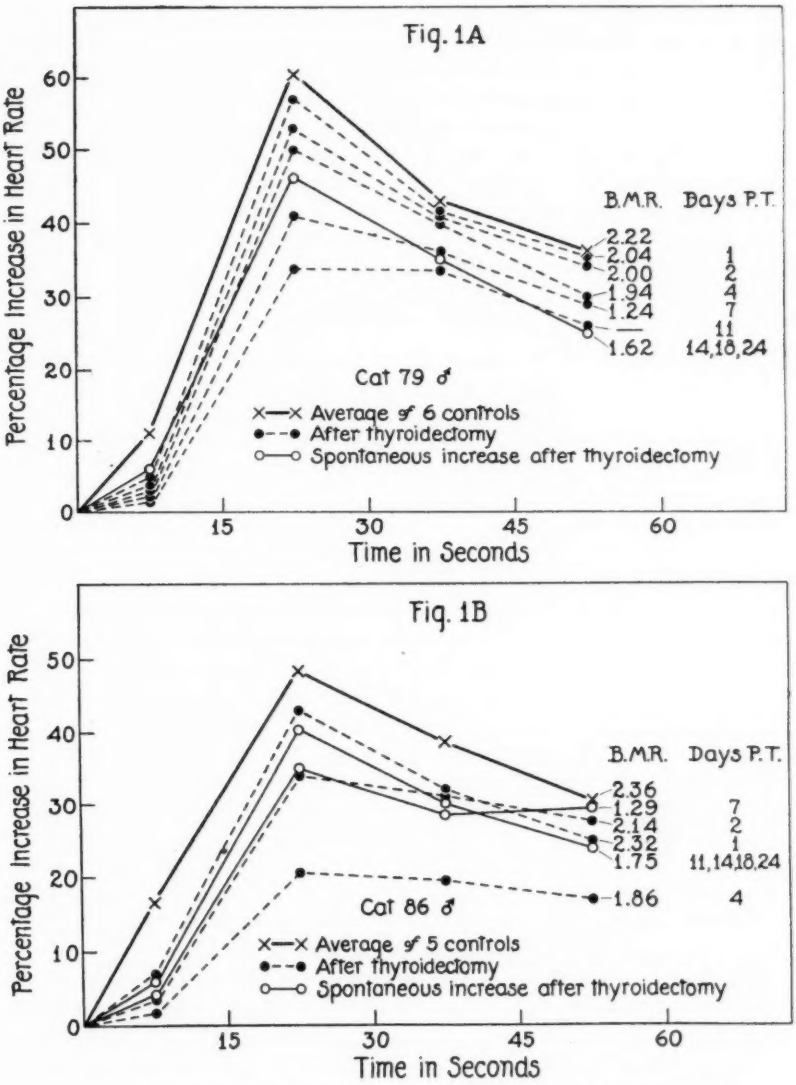

Fig. 1. The response of the denervated heart to a standard dose of 1:500,000 adrenine before and after thyroidectomy in two cats, 79 (A) and 86 (B). Both animals show a partial spontaneous recovery of rate response. *B. M. R.* refers to oxygen consumption in calories per kilogram per hour. *P. T.* means post-thyroidectomy.

about half-way in 13 days, while in two it had only come back half-way in 24 days (fig. 1A and B). In all these animals, the response of the heart also rose. Like the fall in the rate response, the rise was noted several days before the metabolism started to increase. In one cat the rate response came back to its pre-operative level, in two it remained somewhat lower than before operation (fig. 1B), while in one it stayed at a considerably lower level (fig. 1A). Observation of these animals for longer periods was not warranted because irregularities began to appear in the heart rate—a phenomenon interpreted as indicating a regrowth of vagal fibers. The spontaneous late rise in metabolism and heart rate after thyroidectomy can be explained as due either to a regrowth of thyroid tissue, or to an increase in activity of accessory thyroids. The latter explanation seems probable, for Marine (1922) states that in the cat and dog accessory thyroid tissue can be demonstrated in upwards of 90 per cent of animals.

Distinct changes in the quality of the heart sounds accompanied the changes in heart rate and metabolism. Before thyroidectomy the heart responded to adrenine with a definite pounding and snapping sound. After thyroidectomy, usually at the low metabolic level, the pounding and snapping quality of the sounds during the acceleration was absent. After the basal metabolic rate returned to normal, either spontaneously or when forced back by thyroxine, the original quality of the sounds returned. It is interesting that Kunde (1927) states that the first noticeable effect on the heart in the production of experimental hyperthyroidism is an increased force of the beat, and in experimental hypothyroidism a decreased force.

The effect of thyroxine. In two of the thyroidectomized animals a semi-purified thyroxine (Squibb and Sons) was given orally. In one cat two 6-mgm. doses were administered on successive days, and 48 hours later the metabolism had reached its pre-operative level. Simultaneously, the response of the heart to 1:500,000 and 1:5,000,000 solutions of adrenine returned to its original height (fig. 2A). In the other cat two 10-mgm. doses of thyroxine were administered; 48 hours later the metabolism had risen 18 per cent above its previous level. In this cat, injection of 1:500,000 solution of adrenine gave a 10 per cent greater increase than was observed in control experiments (fig. 2B).

1. OBSERVATIONS ON SECRETED ADRENINE. In a study of the interrelationship between the thyroid and adrenals the question arises as to whether the thyroid affects the response to secreted and injected adrenine in the same way. To settle that question the adrenal medulla was stimulated by exposure of the animal to cold of a certain temperature for a given length of time and by the performance of a standard amount of exercise. It had been previously shown by Cannon and his co-workers (1927) that both stimuli cause an increased secretion of adrenine. The chronic denervated heart was again used as an indicator of sensitivity to secreted adrenine

since it could be observed over a period of several weeks and experiments could be made on the same animal before and after thyroidectomy and thyroxine.

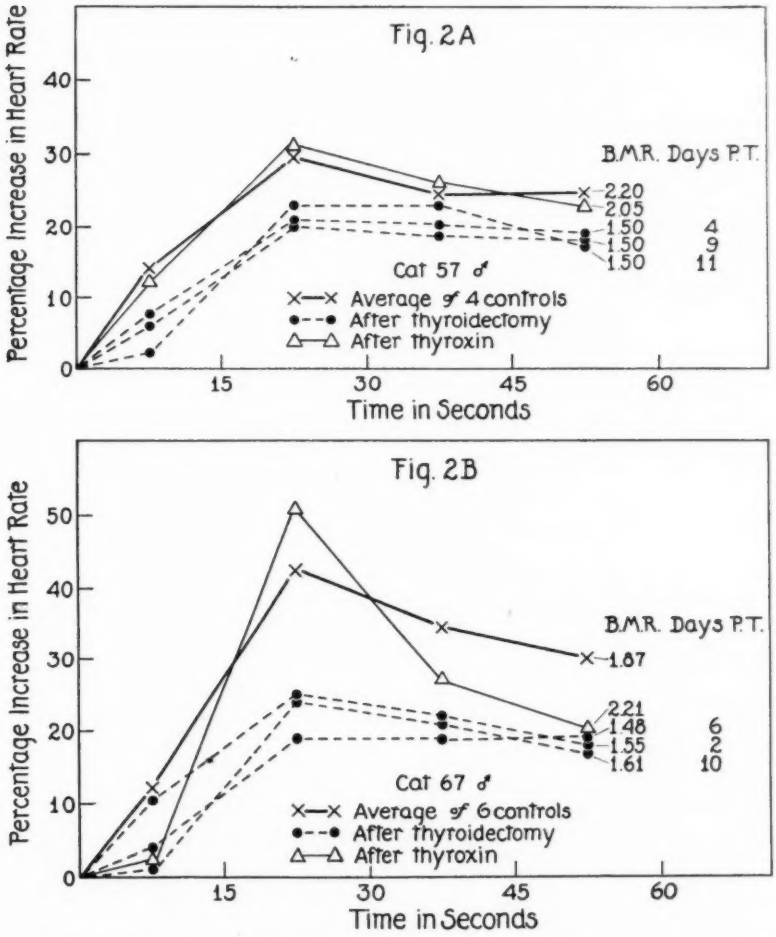

Fig. 2. The response of the denervated heart to a standard dose of adrenine before and after thyroidectomy and thyroxine in two cats, 57 (A) and 67 (B). In graph A the rate response was brought back to normal by 12 mgm. of thyroxine fed orally. In graph B the rate response was raised above normal by 20 mgm.

EXPERIMENTS WITH COLD AS A STIMULUS. After recovery from the heart denervation the animals were trained to sit or crouch quietly in a cage with

a stethoscope strapped to the chest. The tubing from the stethoscope led out through the door of the cage, so that the heart rate could be counted without disturbing the animal. During the experiment the cat was kept

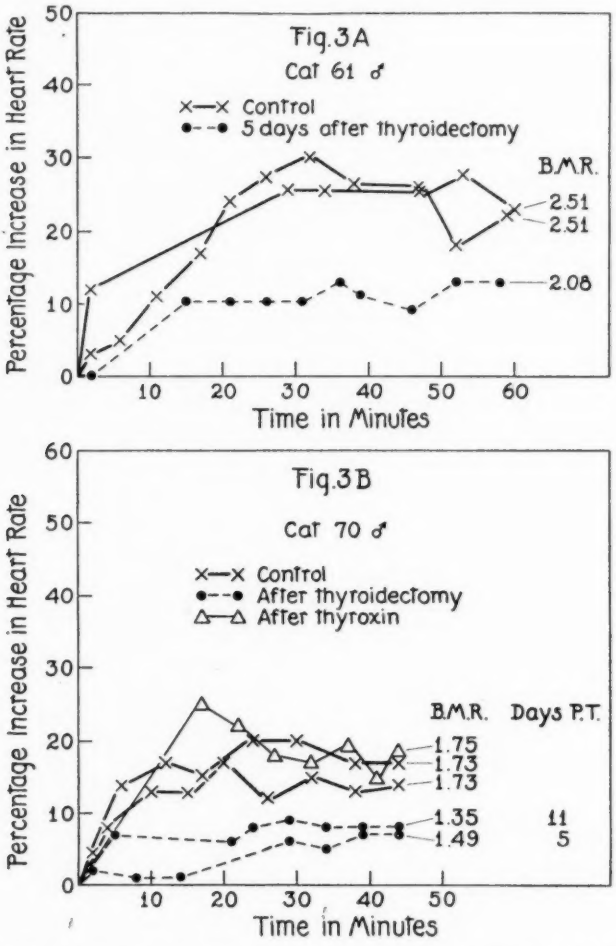

Fig. 3. The response of the denervated heart to standard exposure to cold before and after thyroidectomy in two cats, 61 (A) and 70 (B). Graph B shows in addition the response after 20 mgm. thyroxine.

from falling asleep. The heart rate was counted for periods of one minute, until a constant level had been reached. The cage was then transferred into the cold-room, with care not to disturb the animal. As a rule this

transfer did not affect the cat, and the basal heart rate remained unchanged. The temperature of the cold-room varied from 1.5 to 2.5°C. for different experiments, but it was not allowed to vary more than 0.5°C. during a given experiment. The animals were left in this environment 45 to 60 minutes. Throughout this period frequent counts of the heart rate were made. Erection of the hairs and shivering usually commenced soon after the entrance into the cold-room. If the animal moved, the heart rate was not counted for at least five minutes after the cat became quiet. Thus the effect of emotion or exercise was ruled out as far as possible. Observations were made on 4 animals. As a rule, 10 to 20 minutes after the animal was placed in the cold-room the heart rate reached its maximum and remained more or less constant during the rest of the stay. Two control experiments were made on each animal. In cat 82 the approximate rise over the basal rate was 23 per cent in one experiment and 20 per cent in the other; in cat 61 the rise was 25 and 27 per cent (fig. 3A); in cat 70, 18 and 15 per cent (fig. 3B); and in cat 52, 38 and 32 per cent. These figures show that while the individual response to the standard cold stimulus was quite constant the reaction varied considerably in the different animals.

The effect of thyroidectomy. Removal of the thyroid produced in all animals a decrease in the response of the denervated heart to exposure to cold. In two cats (fig. 3A and B) on the fifth day after thyroidectomy and in another on the sixth day there was approximately a 50 per cent decrease in the rate response. In these three animals there was also a substantial decrease in the oxygen consumption at the same time. In a fourth cat there was no change in the rate response to the cold stimulus on the fifth day after operation. Unfortunately the metabolism was not determined on that day. In this animal, however, on the twelfth and twentieth days the oxygen consumption had fallen and then the rate response had decreased approximately 50 per cent.

A spontaneous return of the oxygen consumption to the normal level occurred in one of the above animals, and accompanying this the rate response of the heart returned to its original pre-operative level.

The effect of thyroxine. In two of the thyroidectomized animals 20 mgm. of a semipurified thyroxine (E. R. Squibb) was fed orally. In one of these (fig. 3B) the oxygen consumption was forced to its pre-operative level and the rate response of the heart to the cold stimulus also rose to its original percentage. In the second cat the metabolism was forced above its original rate, and the accelerations of the heart then rose approximately 10 per cent above the original 20 and 23 per cent rises observed in the two control experiments.

2. Experiments with exercise as a stimulus. Cannon and Britton (1927) have shown that if the adrenals are intact exercise causes an increase in the rate of the denervated heart. In the use of exercise to stimulate the

adrenal medulla in the present study, the stimulus was standardized by employing a treadmill run at a certain speed for a fixed time. This treadmill was a large hollow wheel, 121 cm. in diameter, mounted on a fixed axle. The wooden sides of the wheel were joined at their circumferences by a heavy wire screen, which formed the runway. It was revolved by means of a crank. Since not all cats could be readily trained for the treadmill, certain animals which learned to walk with a regular, even gait after a few preliminary trials were selected for these experiments. Observations were made on 4 cats, all of which walked readily in the treadmill and showed no excitement.

A stethoscope was first strapped to the cat's chest and then the animal was placed in the treadmill cage. After it had assumed a crouching position, the heart rate was counted with a stop watch and recorded every 15 seconds. When the rate had become constant the stethoscope tubing was disconnected, the door of the cage closed and the wheel was revolved. The usual speed was 12 or 16 revolutions in one minute. The same number was always used for a given animal. As soon as the revolutions stopped the cat resumed its crouching position. The stethoscope tubing was immediately reconnected and counting begun. The time between the end of the exercise and the start of counting was always recorded and was usually from 15 to 20 seconds. The rate was counted for two minutes and was recorded every 15 seconds.

In a trained, fasting animal the effect of a standard amount of exercise on the denervated heart was fairly constant. For example, in cat 70, 8 experiments over a period of three days resulted in a maximal rise of 7 to 8 beats in the first quarter-minute period after exercise (fig. 4A). Cat 86, in 11 experiments over five days, showed a rise of 11 to 13 beats for the same amount of exercise. Similar control experiments were obtained on the other animals.

The effect of thyroidectomy. A definite lessening of the response of the denervated heart to the standard exercise was observed after thyroidectomy in 3 cases (70, 72, 61). In cat 61, before thyroid removal, the maximal increase of the denervated heart in the first quarter-minute period after exercise was 41 per cent (average of 6 experiments). Four days after thyroidectomy the increase following the same exercise was 31 per cent, and on the twelfth and thirteenth days it was reduced to 18 per cent. The drop in oxygen consumption during this time was from 2.51 to 1.53 cc. per kgm. per hour. In cat 72 (fig. 4B) the increase before thyroidectomy was 40 per cent (average of 10 experiments). On the fifth and sixth days after operation this had fallen to 28 per cent, and on the tenth and twelfth days to 23 per cent. The fall in basal metabolism was from 2.11 to 1.73 cc. of oxygen per kgm. per hour. In a third animal, cat 70 (fig. 4A) the prethyroidectomy response was an increase of 28 per cent (average of 8 experi-

ments). Five and 6 days after the removal of the thyroid the rate response had decreased to 22 per cent and on the tenth and eleventh days to 15 per cent. The oxygen consumption fell from 1.73 to 1.35 cc. in this time. In the fourth cat (86), instead of a drop, a slight rise occurred in the rate

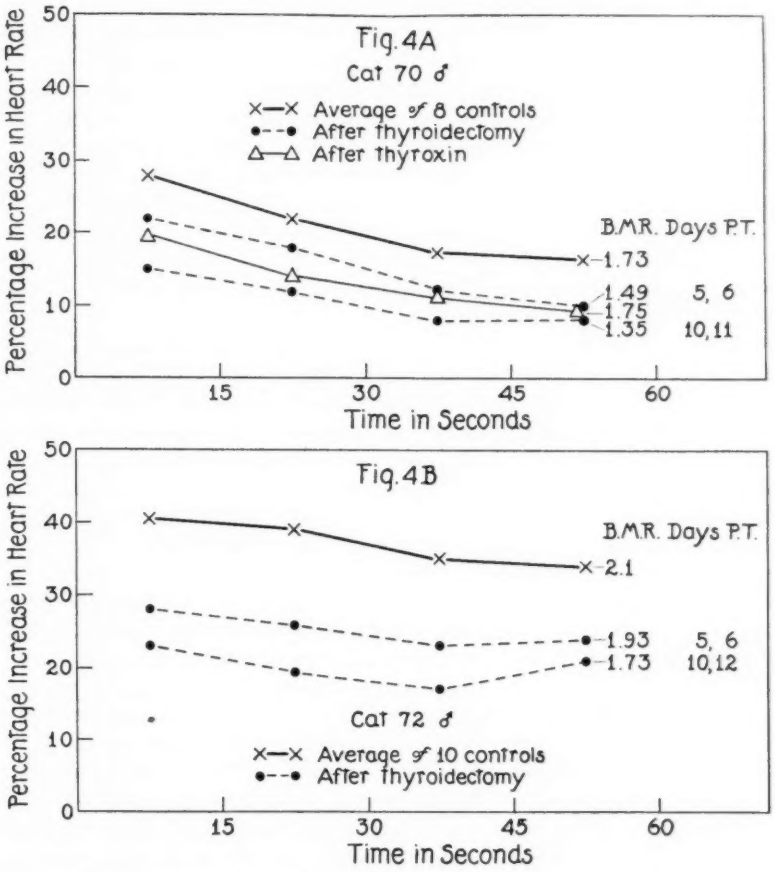

Fig. 4. The response of the denervated heart to standard exercise in two cats, 70 (A) and 72 (B), before and after thyroidectomy.

response of the denervated heart, calculated on a percentage basis. There was, however, in this animal a drop in the rate response from a control of 12 beats to 9 after thyroidectomy. This was more than offset by the very marked reduction of the basal rate from 29 to 23 following removal of the gland.

The effect of thyroxine. Thyroxine was administered orally to 2 of the above animals (70 and 86). In cat 70, after 20 mgm. were given, the metabolism returned to normal, but the rate response of the heart only increased from 15 to 20 per cent. This was considerably below the pre-thyroidectomy response of 28 per cent (fig. 4A). A different result, however, was obtained in cat 86. This animal had shown the least effect from thyroid extirpation. It was given 12 mgm. of thyroxine, and two days later the response of the heart was much more than the control; e.g., the control rise was 40 per cent, but after thyroxine this was increased to 58 per cent. Following a second dose of 6 mgm. thyroxine there was a slight decrease in rate response instead of a further increase, though the response still was greater than the control. The changes in basal rate observed in this animal were interesting. Before thyroidectomy the rate was 29 beats per quarter-minute. On the sixth post-operative day this was reduced to 23. After the first dose of thyroxine the rate rose to 27, and after the second dose to 34.

DISCUSSION. From the above experiments it is obvious that removal of the thyroid decreases the response of the denervated heart to both injected and secreted adrenine. This decrease in rate response is in both cases approximately of the same order of magnitude. Thyroxine, on the other hand, increases the effect of adrenine. The question then arises as to how the thyroid influences the sympatho-adrenal apparatus. Does it sensitize the structures which react to adrenine? Does it increase the output of adrenine from the adrenal medulla? In our experiments on injected adrenine the entire effect of the thyroid must have been on the structures which react to adrenine, since in all cases the adrenal medulla had been removed. If in the experiments on secreted adrenine the output as well as sensitivity was affected, one would expect a greater diminution in the rate response of the denervated heart than in experiments with injected adrenine. The decrease after thyroidectomy, however, was approximately the same with injected and secreted adrenine. The decreased response to secreted adrenine can therefore be explained entirely on the basis of a decreased sensitivity of the heart tissues after removal of the thyroid. The question whether the diminished response to adrenine after thyroidectomy is characteristic of structures other than the denervated heart is not settled.

The specific reduction of the response of the heart to adrenine after thyroidectomy may be of particular interest to the cardiologist, in view of the recent publications on the treatment of advanced congestive failure and angina pectoris by total ablation of the thyroid (Blumgart, Riseman, Davis and Berlin, 1933; Levine, Cutler and Eppinger, 1933).

We wish to point out a few factors which might help account for the relief of pain in angina pectoris, without attempting a complete explanation of

the phenomenon. Starling and Visscher (1926) have shown that the heart uses somewhat less oxygen to do a given amount of work per unit time when the rate is slow than when it is rapid. In other words, slowing of the heart enables it to function more economically. Moreover, as Harrison, Ashman and Larson (1932) have suggested, the slower heart rate is advantageous because the recovery period is prolonged, thus giving the oxygen a greater opportunity to diffuse. Therefore, from both points of view, the experiments indicate that thyroidectomy has an influence favorable to the action of the embarrassed heart, because the diminished rate response to various stimuli necessitates the use of less oxygen and the muscle has a greater opportunity to recover.

Furthermore, Anrep and his colleagues (1926–1933) have shown that the stronger the contraction of the heart the more conspicuous is the diminution of coronary inflow during systole and the greater the back thrust from the coronary artery into the aorta. From the changes in the character of the heart sounds after thyroidectomy, as noted in these experiments (see p. 626), it is suggested that the strength of contraction may be diminished as well as the rate, thus insuring a greater coronary blood supply.

If *lack* of oxygen is the important factor in precipitating attacks of angina pectoris, as suggested by Keefer and Resnick (1928) and Lewis (1932), then total thyroidectomy may alter the cardiac response so that the blood supply to the heart becomes adequate.

SUMMARY

A study was made of the response of the denervated heart to injected and secreted adrenine before and after thyroidectomy and thyroxine. Cats were used exclusively.

In six animals intravenous injection of a standard adrenine solution produced a practically constant increase in heart rate when administered on different days or twice on the same day. Similarly, the response of the denervated heart to adrenine secreted after a standard exposure to cold (4 cats) or to exercise (4 cats) was very constant on different days. After thyroidectomy a lowering of the basal heart rate was observed in most of the above animals, but the maximal effect was not apparent for several days (tables 1 and 2).

Removal of the thyroid gland was followed in all six cats by a 30 to 60 per cent decrease in the rate response of the heart to a standard solution of injected adrenine (table 2 and figs. 1 and 2). In the experiments with secreted adrenine thyroidectomy was followed by approximately a 50 per cent decrease of the heart response to standard cold and exercise stimuli (figs. 3 and 4). In all cases the decrease in response did not reach its maximum for some days.

After thyroidectomy a decrease in oxygen consumption accompanied the decrease in rate response of the heart but usually followed the latter (table 2).

In some animals a partial return of the oxygen consumption and of the rate response to secreted and injected adrenine took place 9 to 14 days after removal of the thyroid (table 2 and fig. 1).

The original response of the heart to both injected and secreted adrenine was restored in several animals by doses of thyroxine which raised the metabolism to its normal level (figs. 2A and 3B). In three animals sufficient thyroxine was given to raise the metabolism above normal, and the response of the heart to injected and secreted adrenine was correspondingly increased (fig. 2B).

After thyroidectomy a change in the quality of the heart sounds in response to injected adrenine was noted in all six animals. In two of these the original quality was restored after the administration of thyroxine.

On comparison of the results obtained with injected and secreted adrenine, the suggestion is offered that the interrelationship that exists between the thyroid and adrenals is due to an altered sensitization of the structures on which adrenine acts rather than to an altered output from the adrenal medulla.

A tentative explanation is given for the relief of pain in angina pectoris by total thyroidectomy.

We wish to express our appreciation to Prof. W. B. Cannon and Dr. S. A. Levine for their suggestions and criticism throughout the course of this work.

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ANALYSIS OF FUNCTION OF A NERVE TO MUSCLE

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The object of this research is twofold. First, it was shown by Eccles and Sherrington (1930) that nerves to skeletal muscle contained two rather distinct size groups of ventral root fibers, and we have generally found that discrete grouping denotes the existence of discrete functions for such groups. We wished to investigate the function of the group of small efferent fibers, since it is obvious that the group of large fibers is motor to skeletal muscle. Second, we wished to test for the various afferent fiber groups and more especially for the relation between fiber size grouping and muscle reflex activity, in the afferent fibers from muscle itself.

In previous communications (Heinbecker, Bishop and O'Leary, 1933, 1934) the nature and function of the fibers in a mammalian skin nerve were discussed. We shall here report similarly on the nature and function of fibers in a typical nerve to muscle. For this purpose we have chosen the nerve to the medial head of the gastrocnemius of the cat. The anatomical approach to the problem was one whereby the various possible sources contributing to the nerve's fiber content could be singly or conjointly eliminated. These sources are the appropriate motor and sensory roots, the spinal ganglia and the ipsolateral sympathetic chain from the diaphragm to the pelvis. In the cat the nerve to the gastrocnemius is derived essentially from the 6th and 7th lumbar and 1st sacral roots. Roots both above and below this level were removed to include certainly the experimental level.

TECHNIC. The animals were operated on under general anesthesia. Section of the roots and removal of ganglia were accomplished after a laminectomy extending from the 4th lumbar vertebra to the end of the sacrum. The roots were cut where the dural extension becomes inseparable from the root trunk. The ganglia were removed with minimal trauma to the motor roots. The sympathetic chain was removed in one piece from the diaphragm to the pelvis. After a sufficient time for degeneration (10 to 20 days) the altered nerves were tested histologically and functionally.

The functional studies consisted of potential analyses of normal sensory and motor roots of the lumbo-sacral region contributing to the nerve to the gastrocnemius, and of the nerve itself, normal, and as modified by the following experimental procedures. 1. Ablation of the 5th, 6th and 7th lumbar, 1st and 2nd sacral spinal ganglia and motor roots. 2. Ablation of the 5th, 6th and 7th lumbar and 1st and 2nd sacral dorsal root ganglia. 3. Ablation of the 5th, 6th and 7th lumbar, 1st and 2nd sacral motor roots. 4. Ablation of the ipsolateral sympathetic trunk from diaphragm to pelvis.

Experiments were carried out in which the amplitude of muscle contraction was correlated with the area of the activity potential in the nerve trunk and with the nerve's fiber content. Analyses of the fiber content of the 3rd, 4th and 6th cranial nerves were made to contrast the results with those obtained from a study of the nerve to the gastrocnemius for purposes that will appear below.

The recording mechanism for nerve studies was the cathode ray oscillograph in association with an amplifier yielding amplification up to 500 mm. per mv. Stimuli consisted of condenser charges with frequencies up to 280 per second available. Myographic recording was carried out isotonically by means of a weighted lever attached to the muscle over a well adjusted pulley; the muscle shortened about 5 mm. for a maximal contraction. The reproducibility of the results and the form of the curves obtained on plotting them testified to the efficacy of the method.

After completion of the functional studies the altered nerves together with the normal controls were divided into upper and lower segments. The upper segments were stretched slightly upon glass rods and fixed by immersion in 1 per cent osmic acid for 12 to 24 hours. After washing for 24 hours they were dehydrated and embedded in paraffin or double embedded in celloidin-paraffin following the technique of Stieve (Romeis, 1928). Sections 5 microns thick were cut from the lower end. The lower segments were drawn through pieces of cat's spinal cord of similar length (Ranson and Davenport, 1931), fixed in ammoniated alcohol and subjected to the Ranson method of silver impregnation. Cross-sections 5 microns in thickness were cut from the upper end. Then silver and osmic cross-sections of the various types of altered nerves and their controls were counted. The diameters of the myelinated fibers were measured and plotted against fiber size.

It seemed desirable to plot these data in a form that would make them directly comparable to the electrical records of the nerves' action currents, since many of the pertinent physiological data on nerve function have been referred to the potential picture. For the graphs of figures 1 to 9, the data were handled as follows. Both threshold and conduction time vary approximately as the reciprocal of the fibers' diameters. A chart was constructed with circles drawn to represent fibers of various sizes, so chosen

that the reciprocals of their diameters differed by whole numbers for simplicity of plotting. This was photographed on a process plate at such reduction that the circles could be compared with nerve fiber sections magnified 500 or 1000 times, either photographed or projected in a dark hood. Millimeters on the glass measuring scale then read as microns in fiber diameter, and the fibers were counted into size groups by comparing each fiber with the nearest measuring circle. Since on this reciprocal scale, equal space intervals subtend smaller size intervals as size decreases, the space intervals for the small fiber range were increased by multiples of those in the large fiber range to correspond to a measurable size difference. The sizes measured were laid off on the base line of the chart, and each size as measured was taken to include fibers from one-half the distance to the next larger size measured, to one-half the distance to the next smaller. The total number of fibers in a group was then divided by the size range (distance on x axis) subtended by that group, to give the amplitude of a rectangle whose area represents the number of fibers in that size group. The step curve is therefore the actual number curve as measured against reciprocal of diameter.

To obtain the approximate curve of area of fibers against reciprocal of fiber size (figs. 3 and 4) for comparison for instance with a plot of action potential area against stimulus strength, the ordinate of each rectangle was multiplied by the square of the average fiber diameter, and plotted over the appropriate fiber size, giving the points for the continuous curves drawn on the charts.

It should be noted, in comparison for instance with Eccles and Sherrington's similar curves plotted directly against fiber diameter, that our manner of plotting alters the apparent distribution, but does not greatly alter the size position of the maxima and minima, although it does alter the relative amplitudes of the elevations as drawn. The area curves are so nearly like the plots of area of action potential against stimulus that they may be substituted for them; in fact if the nerve is large, so that not all fibers are equally accessible to the stimulating current, there is almost certainly a greater error in the recording of action currents than in the counting of fibers. Further, where the picture is that of a potential of large area followed by one of small area, our recording apparatus may be deflected so far by the former, at a sensitivity that would record the latter, that an instrumental distortion is introduced into the measurement of the smaller area, which leaves the anatomical measurement the more reliable index of the nerve's composition. The areas of the two potentials under consideration for the two groups of fibers in deafferented muscle nerves have values to the order of 100 to 1.

1. *Histology of the nerve to the gastrocnemius muscle.* Our fiber counts on muscle nerves confirm those of Eccles and Sherrington. All the myelinated

fibers were counted by size in the operated and normal nerves to the medial head of the gastrocnemius, in three cats with dorsal roots cut, three with ventral roots cut, two with both cut and sympathetic intact, and seven sympathectomized. Total fiber numbers were counted after pyridine-silver for differential counts of non-myelinated fibers, in nerves from operated and normal sides of fourteen of the above cats. Considerable variation occurs in number of fibers, but much less in distribution. The sections for size counts were taken at least 20 mm. from the entrance of the nerve into the muscle to avoid any serious error due to terminal fiber branching.

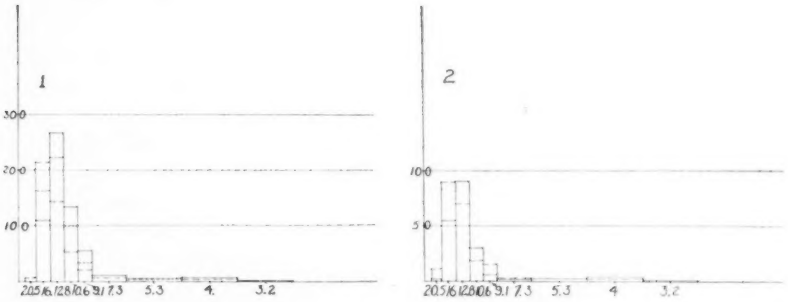

Fig. 1. Nerve fiber size distribution in the trunk to the medial head of the gastrocnemius of the cat. Three groups of normal nerves, each group averaged; from the opposite sides from nerves operated as follows: solid line, 3 deafferented; long dashes, 3 motor fibers cut, short dashes, 7 sympathectomized. The divisions of the abscissa are proportional to the reciprocals of the fiber diameters in μ beneath. The areas of the rectangles are proportional to the numbers of fibers in each group. Further explanation in the text. This distribution corresponds closely to the distribution of action potentials after conduction. Figures on ordinate are such that multiplying the value for the amplitude of any rectangle by the base gives the number of fibers in that group, the 16μ division being taken as unity.

Fig. 2. Like figure 1, but operated nerves. Solid line, deafferented; dash, motor roots cut. For plots of same data on linear instead of reciprocal scale, see figure 6. Numbers of fibers derived by multiplying the amplitude of any rectangle by the base, the 16μ group having unit base.

Figure 1 presents the averages of normal nerves from the unoperated side as follows: ----------, 7 sympathectomized, ————, 3 deafferented, - - - - - - -, 3 ventral roots cut. Figure 2, presents on the same fiber size scale, but at double the amplitude, the data for the operated nerves, the 3 deafferented, the 3 with ventral roots cut. If either operation eliminated one-half the fibers of each group, the corresponding curves of figures 1 and 2 should be identical insofar as the two sides of the cats were originally alike. As a matter of fact, the sum of the sensory and motor alone is less than the normal,

probably in part due to injury in operation to the roots intended to be left intact. In all the *normal* nerves, the plots show a maximum in the range of 16 to 21μ, a second less prominent maximum at 4μ, and in no normal nerve

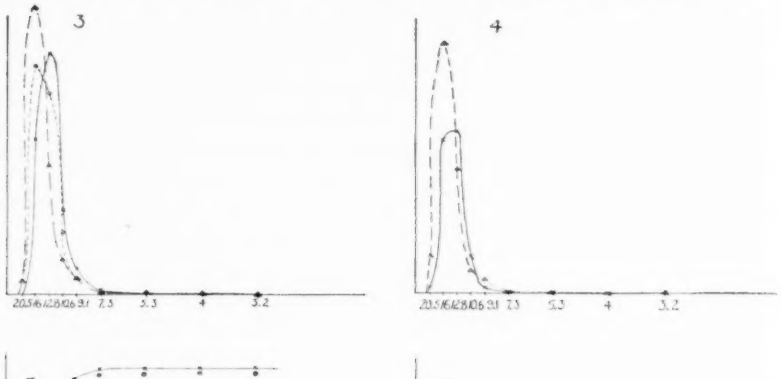

Fig. 3. Plot of the values; amplitude of each rectangle of figure 1 times the square of the diameter of the average fiber, against the reciprocal of diameter of the middle size of that group. Continuous and dash lines as in figure 1. If strength of stimulus is laid off linearly along the base line, the same curve closely fits the potential area values obtained.

Fig. 4. Same as figure 3, but for operated nerves. Solid line, deafferented, dash, motor roots cut. Values along ordinate are twice those of figure 3. See text.

Fig. 5. Cumulative curves of area of fiber cross-sections; 3 upper curves, groups of figure 1, X, normal side of sympathectomized preparation, O, of deafferented, △, of motor roots cut. Two lower curves, groups of figure 2, operated nerves; ● deafferented, ▲ motor roots cut. Plotted against reciprocals of fiber sizes printed below base line.

Fig. 6. Linear distribution curves of fiber number against fiber size, nerves of figure 2. Solid line, deafferented; dash, motor roots cut. For numbers of fibers in each group, see figure 2, since the same groups are plotted there.

was any size group between these empty. In plots of *deafferented* nerves, these two groups are more distinct, one nerve having only 2 fibers in the 9μ group, the others 4 and 7 at 7μ. These extremes are masked in the average

curve. The plots of *sensory* fibers on the other hand show no interval between two groups, but the number decreases progressively from the maximum with decrease of size. This configuration is, to be sure, a function in part of plotting on a reciprocal scale; the *difference* in configuration between motor and sensory components is real, although this difference is accentuated by the manner of plotting. To show this, figure 6 contains the numbers of sensory and motor components plotted linearly from the same data as figure 2. Since the form of the action potential after conduction, or of the curve of action potential area against stimulus strength, has the dimensions of the reciprocal plot of fiber sizes, rather than the linear, the degree of difference shown in these curves may be taken to have the same significance as a similar difference in action potential form or stimulus strength-area curve; at least the anatomical and physiological data appear in comparable terms. To make them more comparable still, figures 3 and 4 give the fiber *area* curves corresponding to the *number* curves of figures 1 and 2, and figure 5 contains the integrals of these, or the cumulative areas, against the reciprocal of fiber size.

In the deafferented nerves, the interval between the two groups of fibers falls at about 8μ. Figure 10 tabulates the number of fibers above and below 8μ, for the three groups of nerves treated above. Corresponding data for the 4th and 6th cranial and for a branch of the 3rd cranial to a rectus muscle are included for comparison, to be considered below.

The data from counts of nerves prepared by the silver technique were handled as follows. It turned out that, due to branching of the nerve trunk near the muscle, one could not be certain that the sections in silver and osmic fixation were comparable; in fact it often occurred that a branch had obviously been lost between these regions. Further, the two sides of the same animal were also not strictly comparable, for the same reason. The total areas of the nerves in osmic were therefore measured, and these divided by the number of fibers counted, to give the average area per fiber (fig. 11). In silver preparations the total including non-myelinated fibers gives a smaller area per fiber. A value for the number of non-myelinated fibers not visible in the osmicated nerves can now be added to the total of myelinated fibers counted in those nerves, such that the area per fiber will equal that for the corresponding sections stained with silver, allowing for 20 per cent shrinkage of the silver-treated nerves as compared with those treated with osmic. The number so added is an approximation to the number of non-myelinated fibers in the osmicated nerves in question; at least the relative values should be significant in similar nerves treated identically. The ratio of area of nerve to area of fibers after fixation has been found fairly constant in the depressor nerve of the rabbit (O'Leary, Heinbecker and Bishop, 1934). Figure 11 is a table of the resulting values. The first line of this table presents the averages for the 3 pairs of nerves

in which the motor roots were cut, the next line that for 3 deafferented, the third for 7 sympathectomized, and the fourth, data on one nerve in which both roots were cut leaving the sympathetic intact. In the second part of this table are actual counts of one pair of nerves of line 3 above, of which the silver preparation was believed to show myelinated fibers clearly enough to distinguish them from non-myelinated. The tendency here would be to count some of the small myelinated fibers as non-myelinated; on the operated side fewer myelinated fibers were indeed found in the silver preparation than in the osmic, while on the normal side, the wider discrepancy is doubtless due to loss of a branch between osmic and silver sections.

Comparing the first two columns of the first part of the table, the loss of about one-half the myelinated fibers in the osmicated nerves, either motor or sensory, has about doubled the area per fiber, while the loss of the sympathetic has caused no significant difference; practically no myelinated fibers enter from the sympathetic. In the silver preparations, the loss of the ventral root fibers does not show, but the loss of dorsal root fibers nearly doubles the average area per fiber. From this we conclude that most of the non-myelinated fibers to muscle from the roots are from the dorsal root. The loss of sympathetic fibers also lies within the error of computation, indicating few fibers from this source to muscle nerves. The per cent of fibers non-myelinated in the normal nerves, 15 to 25 per cent of the total, corresponds reasonably considering the approximate character of their estimation, with the direct count of 34 per cent (probably too high), and give ratios myelinated to non-myelinated of 1:0.18 to 1:0.33, and 1:0.52 for the one counted nerve, comparing with Davenport and Bothe's (1934) ratio of 1:0.77 for the L_5 dorsal root of the human with few such fibers in the ventral root at this level, ratio 1:0.07. The estimates for the per cent of non-myelinated fibers increases in the nerves with ventral roots cut, but increases less in those with dorsal roots cut, again indicating that the majority of non-myelinated fibers were from the dorsal roots; while after sympathectomy a slight increase, presumably within the error of computation, indicates again that relatively few fibers enter from the sympathetic. The latter finding is confirmed by direct counts in part 2 of the table, where loss of non-myelinated fibers after sympathectomy was one-tenth the total.

These values, derived as they are by a round-about calculation, are admittedly inexact, but are presumably reliable as indicating the *relative* number of fibers entering the motor nerve from the various sources. The obvious sources of error are systematic ones, affecting the various nerves similarly; for instance, the relative amounts of shrinkage in osmic and silver techniques.

2. *On the functions of the two size groups of motor fibers.* As will appear below, practically all the tension of the gastrocnemius is developed upon

stimulation of only the first group of large motor fibers. It was impossible to decide, on the basis of the physiological experiments to be presented, whether any tension at all was assignable to the second group, since it might be that these small fibers had an effect too weak to be detected in the

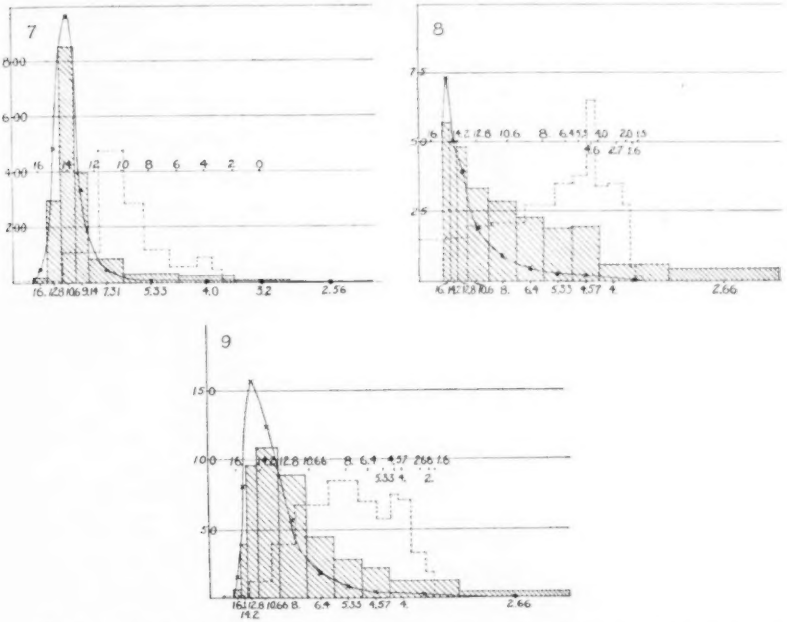

Fig. 7. Fiber distribution plots of branch of third cranial nerve of cat, to rectus muscle. Solid line, rectangles, fiber number against reciprocal of diameter as in figure 1. Dash line, fiber numbers against linear function of diameter as in figure 6. Smoothed curve, area of fibers against reciprocal of size as in figure 3. Note that third nerve distribution of large fibers is nearly identical with that of the nerve to the gastrocnemius in contrast to those of the fourth and sixth nerves following; but it shows no second maximum in the small fiber range in reciprocal plots, and less, even in the linear plots, than the nerve to the gastrocnemius.

Fig. 8. Fourth cranial nerve of cat, as in figure 7. While showing the sharp maximum of figures 1 and 6, this nerve tapers off to the smaller sizes much more gradually, in such a manner as to obscure the separation out of a second group of small fibers if such exists.

Fig. 9. Sixth cranial nerve of cat, as in previous figures. Again as in the fourth nerve, many medium sized fibers occur, with no sign of a second group.

presence of the larger effect of the larger fibers. It occurred to us that the smaller group might be motor to muscle spindles, but no clear-cut method of demonstrating this directly has been devised. It is stated, however, by

Dogiel (1906) that the extrinsic muscles of the eye have no muscle spindles. Tozer and Sherrington, investigating cat, rabbit and monkey eye muscles, found, especially in the latter, numerous structures which they believed to be receptive muscle spindles, though of simple form. They do not state

FIGURE 10

CONTROL NERVE					OPERATED NERVE				
Nerve	No. above 8μ	No. below 8μ	Total	Per cent below 8μ	Nerve	No. above 8μ	No. below 8μ	Total	Per cent below 8μ
Motor out					Motor out				
6	391	153	544	28.1	6	136	43	179	24
7	602	305	907	33.6	7	247	84	321	26.2
8	321	176	497	35.4	8	206	144	350	41.2
Aver.	438	211	649	32.5	Aver.	196	90	286	31.5
Sensory out					Sensory out				
11	470	194	664	29.2	11	191	106	297	35.7
16	554	347	901	38.5	16	100	90	190	47.3
17	754	401	1155	34.7	17	175	123	298	41.3
Aver.	593	314	907	34.6	Aver.	155	106	261	40.6
Sympathectomized					Third nerve				
1	544	192	736	26.1	Cat no. 20	1560	471	2031	23.2
4	485	175	660	26.5	Fourth nerve				
5	598	165	763	21.6					
10	882	623	1505	41.4	Cat no. 15	433	367	800	45.9
12	441	179	620	28.9	Sixth nerve				
13	445	403	848	47.5					
9	349	106	455	23.8	Cat no. 15	867	599	1466	40.8
Aver.	535	263	798	33.0					

Fig. 10. The division at 8μ separates two rather distinct groups of fibers in deafferented nerves to the gastrocnemius, not present in those with motor roots degenerated (in reciprocal plots). The less clear division shown in the plots of normal nerves is, therefore, due entirely to the motor component, and is only obscured by the sensory component. The third cranial nerve, although comparable to the above in large fiber distribution, shows a small fiber distribution and number below 8μ comparable to the gastrocnemius nerve with the small afferents present, but with the small motor group omitted. The fourth and sixth nerves, although showing no second group, have large numbers of fibers below 8μ in contrast to the smaller numbers of the third nerve. However, this increase is largely in the middle range, 5 to 8μ.

the relative numbers of such structures, nor do they refer to motor endings on them. In this state of affairs, it might be that the nerves to eye muscles would contain no second group of motor fibers. We therefore counted all the myelinated fibers in the 4th and 6th cranial nerves of one cat, and in one

branch each of the 3rd cranial of two cats, into size groups as shown in figures 7, 8 and 9.

FIGURE 11

Estimates of non-myelinated fibers in normal and operated nerves

A

CONTENT	AVER. AREA FIBERS OSMIC × 10^{-8} mm.^{2}		AVER. AREA FIBERS SILVER		NOS. MYELINATED COUNTED AND TOTAL FIBER ESTIMATE IN OSMIC		PER CENT NON-MYELINATED OF TOTAL		RATIO OF MYELINATED TO NON-MYELINATED	
	Normal	Operated	Normal	Operated	Normal	Operated	Normal	Operated	Normal	Operated
Sensory + sympathetic					649	287	25.7	64.1	1:0.35	1.80
Av. 3 nerves	260	550	193	194	875	803				
Motor + sympathetic					908	270	15.1	26.0	0.18	0.35
Av. 3 nerves	191	400	163	296	1070	365				
Motor + sensory					886	727	20.2	25.6	0.25	0.26
Av. 7 nerves	183	197	147	146	1110	980				
Sympathetic only	180	4710	147	342	1129	14	18.7	92.8	Not signif.	
1 nerve					1388	193				

B

ACTUAL COUNT	TOTAL MYELINATED OSMIC	TOTAL MYELINATED SILVER	NON-MYELINATED SILVER	TOTAL SILVER	PER CENT OF TOTAL NON-MYELINATED SILVER	RATIO MYELINATED TO NON-MYELIN.
1 nerve normal	736	581	304	885	34.3	1:0.52
Sympathectomized	733	719	319	1038	30.7	1:0.44

Fig. 11. *A*. The area of each nerve was divided by the number of fibers, myelinated and total, to give the average area of nerve per fiber, showing in osmicated and silver cross sections, first four columns, 20 per cent greater shrinkage in silver than in osmic was allowed for. In the fifth and sixth columns, numbers have been added to the counts of myelinated fibers, sufficient to decrease the average areas in the first and second columns to those in the 3rd and 4th respectively. The upper figure is the osmic count, the lower, the total estimate. The numbers so added are the presumptive non-myelinated fibers present, but not stained in the osmicated sections. These numbers are the basis for the computations of per cent and ratios in the last columns.

B. Counts of myelinated fibers in osmic and of myelinated and non-myelinated separately in silver of one especially clearly stained nerve of line 3, above.

These nerves were not deafferented (probably an impracticable procedure), and it is not known how many afferents may run in them, but they presumably contained the whole afferent supply to the muscles they innervated (Tozer and Sherrington, 1910). Since the 3rd nerve branches were

necessarily short, the sections were taken from within a few millimeters of the muscle; whether terminal branching will account for some of the small fibers present is not known. Eccles and Sherrington did not find that this obscured the division into two groups in other motor nerves. At any rate these two factors, afferent supply and motor branching, might be considered to account for at least as many fibers below 8μ, as occur in the afferent gastrocnemius supply after ventral root section. We may then compare the size distribution in the motor nerves to eye muscles with the three distributions to the gastrocnemius, the normal nerve, the afferent component and the motor component.

In 13 normal nerves, the per cent of fibers below 8μ ranged from 21 to 47, with an average of 33. For 3 sensory components the average is 31.5, for 3 motor, 40.6 (fig. 10). For the 3rd nerve branch, the figure is 23, for the 4th nerve, 46 and for the 6th nerve, 41. Of these, only the 3rd nerve has a distribution of large fibers comparable to the gastrocnemius supply; here the percentages of small fibers are unequivocably smaller than the average. If the range below 6μ is considered, a greater discrepancy exists; that is, while the gastrocnemius has its fewest fibers at 8μ, the numbers in the 3rd nerve are still rapidly decreasing in this range (see also linear plots). If the fibers below 8μ are presumed to be all sensory, and therefore, to be compared with the total sensory component plus the motor component above 8μ of the gastrocnemius medius, the two nerves have about the same ratios, 23 per cent as compared with 20 per cent. One may conclude then, either that the 3rd nerve lacks the second group of motor fibers, or that it has a slight representation of small sensory fibers (or partly both).

The form of the distribution curve is indicative of the first conclusion. On the reciprocal plot, there is no sign in the 3rd nerve of a division at 8μ or anywhere else, as there is in the normal gastrocnemius. It has the form that would be obtained by adding to the gastrocnemius sensory component the large fiber group of the motor. The 4th and the 6th nerve distributions are not so critical, since it might be assumed that the apparent spread of the large fiber group toward the smaller size had obscured an interval if it existed. We do not know whether the oblique muscle which the 4th nerve innervates has muscle spindles.

Pending further inquiry we may infer that the second motor group is absent or small in the oculomotor nerve, and its presence in muscles containing muscle spindles may account for their motor innervation.[1] Except-

[1] There exists histological evidence that the motor fibers to muscle spindles of the cat are small fibers, distinct from motor fibers to other elements. Ruffini (1898) found that they were the smallest fibers of the three sizes going to muscle spindles, and that they passed, one to each spindle fiber, from the nerve trunk without branching, while fibers to contractile muscle elements did not send branches to muscle spindles. Creed, Denny-Brown, Eccles, Liddell and Sherrington (1932) state that the motor end plates of spindle fibers are supplied by small unbranched medullated

ing what slight tension these muscle spindles might exert in the whole muscle, not detectable in our records, and presumably less per fiber than the tension of the primarily contractile fiber, this corresponds to the finding to be reported below that the total effective pull of the muscle follows the stimulation of only the larger group of fibers. It is not consistent with the inference of Blair and Erlanger (1933), that since fibers of the ventral root range from 20μ down to 2μ, the *functional motor* range is similar. The fibers mediating other functions they presumed to range as widely in their conductivity, which, they apparently thought, implied an exception to our belief that unit functional modalities are usually rather strictly confined to the fiber size groups that appear in the analysis of mixed nerves. The nerve to the gastrocnemius, containing a group of fibers to which its typical contraction function can be assigned, shows the usual range of such groups, i.e., a range of 1:2 or 3 in fiber size.

Eccles and Sherrington divided the total tension of the muscle by the total number of efferent myelinated fibers to obtain the average tension per motor unit. If the functions of the motor groups are different, a distinction might be made between the tension per motor unit involving the ordinary contractile fibers, and that of a unit involving muscle spindles. The latter is presumably much smaller, the former somewhat larger than the average at which they arrived.

3. *Potentials of afferent and efferent components to muscles.* Potential analyses were made of normal sensory and motor roots of the lumbo-sacral region, of nerve trunks to the gastrocnemius muscle, both normal and those modified by the following experimental procedures. 1. Ablation of the 5th, 6th and 7th lumbar, 1st and 2nd sacral spinal ganglia and motor roots. 2. Ablation of the 5th, 6th and 7th lumbar, 1st and 2nd sacral dorsal root ganglia. 3. Ablation of the 5th, 6th and 7th lumbar, 1st and 2nd sacral motor roots.

The conducted neurogram of the 2nd sacral dorsal root shows a main potential elevation with a conduction rate of the fastest fibers varying from 110 to 80 meters per second. This potential is followed by a second lower potential seen as a definite potential maximum with a conduction rate of 35 to 25 meters per second. Its threshold is 4 to 5 times that of the most irritable fibers. It corresponds to the B_1 potential of the saphenous nerve (Heinbecker, O'Leary and Bishop, 1933). Blair and Erlanger (1933) have attempted to explain the failure of Erlanger and Gasser (1930) to find a B wave in mixed nerves after stimulation of the dorsal roots, on the presumption that the fibers are regrouped, or redistributed, in such a way that a potential of indefinite form in any one root might emerge as a definite wave

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fibers. Hinsey however (1927) quotes other authors that in Ophidian and Saurian muscles, motor branches from fibers to skeletal muscle also innervate muscle spindles.

in the saphenous. This seems to be another way of saying that not all roots contain a distinct group of B fibers, which may be correct. The region between A and B sizes in the roots also contains many afferents to muscle, which, while not eliminating a potential in the B range, renders it less distinct from the A in the root itself. In the S_2 root there is present also a low C potential with a high threshold, a conduction rate of 1 to 0.4 meter per second and a long absolutely refractory period.

The conducted electroneurogram of the excised lumbar and sacral motor roots show a typical A elevation with a maximum amplitude elicited by a stimulus approximately 3 times that of the most irritable fibers. A low tail is added to the main elevation by increasing the stimulus strength 4 to 6 times the threshold value but the increase in total potential area is insignificant. The conduction rate of the fastest fibers averages from 110 to 80 meters per second, that of the fibers giving rise to the tail cannot be accurately measured but probably ranges down to 20 or 15 meters per second. The absolutely refractory period of the whole of the A potential including the tail is short, 0.7 to 0.9 sigma. In a few preparations a low potential traveling at a rate varying from 12 to 8 meters per second with a threshold 15 to 20 times that of the most irritable fibers has been observed. A potential corresponding to this has been studied in visceral nerves of the cat (Bishop and Heinbecker, 1930) and originates in myelinated fibers of the autonomic type. In the electroneurograms of many of the motor root preparations a low C potential is present. This is derived from the non-myelinated fibers present in such roots. The number of autonomic fibers in these motor roots is so small that their detection by means of the potential record is possible only in the best preparations.

The conducted action potential of a normal nerve trunk to the gastrocnemius muscle has a first elevation followed by a tail which does not appear as a definite maximum. From most nerves a low C potential is elicited. A B_2 potential has not been recognized here. This probably corresponds to the fact that there are few, if any, post-ganglionic myelinated autonomic fibers in nerves to muscle. The maximum amplitude of the A potential is obtained with the stimulus strength $2\frac{1}{2}$ to 3 times the threshold value for the most irritable fibers. In many preparations no further increase in area is seen no matter how strong the stimulus. In preparations in which the whole sciatic nerve is stimulated and the recording leads only are on the small nerve trunk to the gastrocnemius, the threshold range from the most irritable fibers to the least irritable fibers causing an increase in potential area is from 1 to 4 or 6. The discrepancy between this figure and that for a smaller branch of the sciatic is apparently due to differences in proximity to the stimulating electrode of fibers of a given size in such a large nerve trunk. The curve of contraction height against stimulus strength has a similar range. It is evident from a study of the fiber size

and area charts that no detectable increase is to be expected in potential area obtained on stimulating the small fibers ranging from 5μ down in these nerves, since they amount to not over 1 per cent of the area of the whole nerve. The slight widening of the main A elevation near its base is presumably due to activity in myelinated fibers of the middle size range.

The potential record of the nerve from animals with ablation of the 5th, 6th and 7th lumbar and 1st and 2nd sacral spinal ganglia shows a first elevation with a tail similar to the normal nerve, with its amplitude and total area reduced approximately one-half. A low C potential is occasionally visualized.

The potential record of the nerve to the gastrocnemius after removal of the 5th, 6th and 7th lumbar, and the 1st and 2nd sacral motor roots shows a main elevation with a tail similar to the normal. There is a reduction in amplitude and area to approximately half the normal value. A "C" potential approximating the normal is usually elicited. Nerves in which the sympathetic chain has been removed as well as the motor roots are similar except for a lower C potential.

Absolutely refractory period determinations on the altered nerves studied showed no indication at all of a wide range of values such as recently described by Blair and Erlanger (1933 *loc. cit.*) for the range of A fibers. Such values have never been found by us except in preparations of nerve fibers damaged by anoxemic conditions or otherwise, and they are regarded by us as being abnormal. Heinbecker (1929) has shown that before the action potential of an anoxemic nerve fiber can no longer be elicited its absolutely refractory period increases approximately 300 per cent, and that of the smaller fibers increases faster than that of the larger. Such a range would cover the values given by Blair and Erlanger for supposedly normal fibers. However, Blair and Erlanger's results do not validly denote a range of absolutely refractory periods even such as they have plotted for them. They derived refractory periods for fibers of 27 m.p.s. and 6.7 m.p.s. conduction rates respectively, by comparing the intervals between shocks, the second shock being 13 times threshold for the fast fiber, and only twice threshold for the slow one. They are, therefore, comparing the absolutely refractory period of the fast fiber with some point out in the relatively refractory period of the slow fiber. More significance can be placed upon the fact that their fastest fibers show refractory periods of 1.25 sigmas instead of the customary 0.90, for the temperature at which they attempted to determine them.

In general then, the potentials from these nerves and nerve components correspond to the fiber distribution as determined histologically on the basis of the usual rules that the larger the fiber, the lower its threshold, the faster its conduction and the greater the area of its potential. In particular, the number of small fibers is not sufficient to add any precision by these

methods to the relationship of various functions to size. In the motor fiber range, the histological measurement probably gives a more precise picture of the details of fiber distribution than can be obtained from action potential analysis.

4. *Efferent fibers responsible for muscle contraction.* The fiber analyses of the trunks to the gastrocnemius muscle from which the sensory compo-

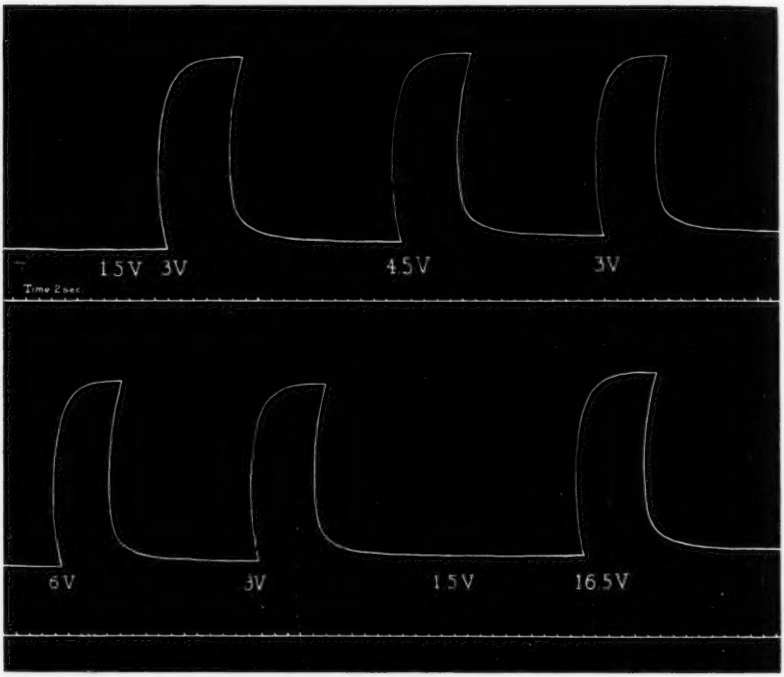

Fig. 12. Myogram of gastrocnemius muscle. Stimulus to nerve containing motor fibers only; interrupted tetanic contractions. Note threshold is just 1.5 volts. At three volts a maximum contraction is secured. Slight variations in amplitude with repetition of this and other stimulus strengths due to differences in duration of rest intervals, and to persistent fatigue when these are short. Lower strip of record continuous with upper.

nents were eliminated show a definite division on the basis of fiber diameter into two groups (fig. 2). Since such a fiber grouping in other nerves has been found to have a functional significance (Bishop and Heinbecker, 1932; Heinbecker, Bishop and O'Leary, 1934), efforts were made to determine the limits of the fiber size range of those fibers responsible for the complete tetanic contraction of a muscle. In principle, the experiment

consisted of the coincidental recording to the electroneurogram and the isotonic-myogram. The nerve trunks to the gastrocnemius of the cat can be separated from the main sciatic nerve for a distance of 5 to 7 cm., giving an adequate length of nerve trunk for both stimulating and recording by means of a specially constructed electrode elsewhere described (O'Leary, Heinbecker and Bishop, 1934). The nerve supply to all other muscles was severed and the blood supply of the limb carefully preserved. The nerve was stimulated at frequencies varying from 45 to 100 per second with no difference in the results except amplitude of tetanus. Care was taken to stimulate the nerve trunk at least 2 cm. from its entrance into the muscle

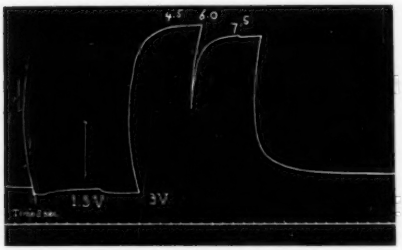

Fig. 13

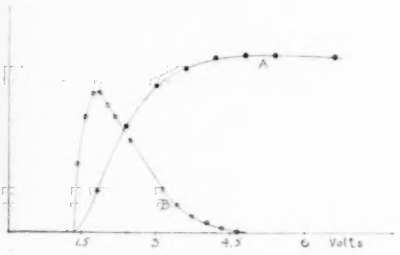

Fig. 14

Fig. 13. Myogram of gastrocnemius muscle on stimulating nerve containing motor fibers only. No increase in contraction amplitude above 3 volts, 1.5 volts threshold for largest nerve fibers. The breaks in the curve are due to intervals in changing from one stimulus strength to another. The decrease in amplitude at higher voltages was accompanied by a depression in amplitude of the electroneurogram simultaneously recorded, and was apparently due to fatigue from rapid stimulation. Stimuli 90 per second.

Fig. 14. *A*. Plot of area of potential record against strength of stimulus, deafferented nerve to median head of gastrocnemius taken immediately after records of figures 12 and 13.

B. Plot of increase of area of potential against increase of stimulus, differential curve of above, for comparison with curves of cross-sectional area against fiber size of figures 1 and 2.

to avoid complications from fiber branching (Eccles and Sherrington). The results are shown in figures 12 and 13. It was found that tetanus of maximum amplitude was obtained by stimulus strengths approximately $2\frac{1}{2}$ times the threshold value for the largest fibers in the nerve trunk. The diameter of the largest motor fibers found by us was about 20μ. On the basis of previous evidence as to the relationship of fiber size to threshold (Bishop and Heinbecker, 1930) with largest fibers of a diameter of 20μ, a stimulus $2\frac{1}{2}$ times threshold would not activate fibers under 8μ. Stronger stimuli up to 8 to 10 times the threshold stimulus failed to produce any

recognizable increment in contraction height. It can, therefore, be said that if the small fiber group (average diameter 4μ), representing approximately 25 per cent of the total number of fibers has any effect on the contractile fibers of the muscle it is too slight to be recognized by the method of recording employed.

The areas of the electroneurogram for the various stimulus strengths were measured by means of a planimeter from monophasic potentials and after killing and blocking between electrodes with cocaine. The potential area increased over the range of stimuli indicated in figure 14. No measurable increase in potential area was obtained from activity of the small fiber group. The sum total of the cross-sectional area of all the fibers of this group is approximately 1½ per cent of the total area of all the fibers in the nerve trunk. With amplification up to 200 mm. per mv., the effect of these fibers is not recognizable. With higher amplification the choking of our resistance coupled amplifier by the first potential distorts the picture. We can only say then, on the basis of these experiments, that the potential area and the tension, plotted against stimulus strength, have a similar form within the limits where we can measure them.

Eccles and Sherrington suggest that the larger motor fibers branch in the periphery to innervate more muscle fibers per nerve fiber than the smaller, that is, the motor units vary in size with size of fibers. Our evidence confirms this, in that it shows that potential area, as a function of fiber area, parallels contraction, but more precise recording by smaller steps of stimulus strength will be required to test this relationship precisely. It may be pointed out that if contraction per motor unit is proportional to size of fiber, a range of tension per impulse per motor unit is provided within the large fiber group (20 to perhaps 6μ) of 10 to 1, while to include all the myelinated efferent fibers to muscle as supplying motor units would give a range of at least 100 to 1. It would further leave no motor fibers for the motor supply of muscle spindles unless, to be sure, these are also included as motor units comparable to the typical motor fibers.

5. *Sensory afferents in muscle nerves.* The action potentials of muscle nerves after degeneration of the ventral roots do not show the sharp division into waves that characterize skin nerves. The main elevation seems to consist of two parts, closely confluent, like an alpha and beta of skin nerves, the latter of which extends into a tail as the stimulus is raised, without a separate elevation to correspond to the sensory B wave of the saphenous. Nevertheless, sensory tests indicate that the sensations are distributed according to fiber size here as elsewhere into definite ranges. The anatomical analysis of such nerves shows no separate group of small fibers, even in the number curve, when plotted on a reciprocal scale of fiber size to compare with potential records, and the area curve reduces their prominence even more, showing merely a falling off of the curve from

the maximum, which lies in the 12 to 16μ range. In linear plots of number against size, there appears a second hump in the distribution in about the same range as the second motor group, but somewhat less prominent. We cannot say, therefore, whether the fibers of 5μ and smaller diameter have potentials in the B position of skin nerves where a distinct elevation is visible because of the relatively more numerous fibers.

The sensory functions of the afferent fibers in a nerve to muscle have been determined partly by animal experimentation and partly by inference from previous experimentation on the function of the afferent fibers in skin nerves taken in conjunction with our knowledge of sensation as a whole. It has been previously shown that direct stimulation of skin nerves fails to produce the sensation of pressure (Heinbecker, Bishop and O'Leary, 1934). Thompson and his associates (1934) recently have questioned the absence of pressure fibers from skin nerves but their presence in muscle nerves is not doubted by these investigators. Clinical experience has established that pressure on muscle yields a sensation of pressure and, if sufficiently great, of pressure pain. It has also been shown by us that on subarachnoid injection of novocaine the sensation of pressure is lost just prior to tactile sensation, the time interval being so slight that the fibers are doubtless of similar size. Inamuch as skin tactile sensation results from activity in the largest myelinated nerve fibers in a skin nerve it is inferred that pressure sense is the result of activity of fibers of similar size in the nerve to muscle and subcutaneous tissues.

Pain results from the activity in small myelinated fibers in skin nerves. In man pressure pain is lost after subarachnoid novocaine injections coincidently with superficial skin pain. It is, therefore, justifiable to consider that small myelinated fibers of nerves to muscle convey the sensations resulting in pressure pain in muscle. Animal experiments in which the potential of a nerve to muscle was observed coincidently with the reactions of an animal, with the nerve still functionally attached centrally, showed definitely that reflex responses such as accompany painful stimuli followed the activation of fibers having a threshold 4 to 6 times that of the most irritable fibers in the nerve. Elsewhere it has been shown that fibers of this relative threshold in skin nerve are myelinated and measure from 6 to 3μ in diameter.

6. *Non-sensory afferent vaso-motor fibers in muscle nerve.* Experiments were conducted to determine the presence and size range of afferent vaso-motor fibers in the nerve to the gastrocnemius. The systemic blood pressure was recorded manometrically simultaneously with the action potential of the nerve centrally attached. Stimuli up to 90 per second were employed. On stimulation up to twice the threshold stimulus for the largest fibers in the nerve a slight (2 to 3 per cent) drop in blood pressure occurred. This could not be changed to a rise with increasing frequency of stimula-

tion, indicating that the fibers being stimulated were exercising a purely depressor effect. Stronger stimuli 4 to 5 times the threshold strength caused a rise in blood pressure (2 to 3 per cent). It is evident, then, that in a muscle nerve there are two regions of the afferent fiber size range having vasomotor reflex effects. On the basis of stimulus strengths in relation to the threshold stimulus for the largest fibers in such a nerve it is calculated that the larger depressor fibers vary in size from 14 to 7μ approximately, and the pressor fibers are of the pain fiber range, 6 to 2μ. Such fiber groups have been demonstrated in skin nerve and in the depressor and vagus nerves of the cat by Bishop, Heinbecker and O'Leary (1934), and their size range approximately determined. It is at least possible that here and in skin nerves the fibers concerned also serve other reflexogenic or sensory function, although in the depressor they are apparently non-sensory.

Reflex effects on respiration were not obtained until the stimulus strength was adequate to stimulate fibers in the pain fiber range and then their effects were slight (2 to 3 per cent). The effects registered were changes in rate and depth.

7. *Proprioceptive and nociceptive fiber groups governing somatic muscle reflex phenomena.* To investigate the fibers concerned in the coördination of the muscular system by reflex action, the nerve from one head of the gastrocnemius was stimulated and its action potential recorded while the nerve trunk to the other head was left intact so that a record of the reflex activity of the gastrocnemius was still obtainable (fig. 15). On stimulation of the larger fibers up to twice the threshold strength, reflex inhibition resulted, provided the muscle was exhibiting some measure of muscle tone. On stronger stimulation fibers were activated which overcame the inhibitory effect of the larger ones and if the stimulation was of sufficient duration and intensity reflex contraction resulted (fig. 15). It is important to note that at a stimulus strength just above threshold, the reflex action was purely inhibitory at any frequency. The amount of inhibition was increased by increases of either strength or frequency of stimulation. No reversal of effect was obtained by prolongation of the period of stimulation or by increasing the rate of stimulation from 45 to 250 per second. At higher stimulus strengths when both inhibitory and excitory fibers were being stimulated, increasing the rate of stimulation caused the excitation effects to set in earlier and to overcome more rapidly the inhibitory effects. On rapid strong stimulation the inhibitory effects as evidenced by activity in the muscle could often be completely prevented, though, of course, centrally their effects were presumably still present. Here as in the case of the vasomotor centers (Bishop, Heinbecker and O'Leary, 1934), there is probably a difference in the order of temporal summation such that increasing the frequency of the excitatory stimuli results in a greater summation of

effect than does increasing the frequency of inhibitory stimuli. Changes in the level of muscular tone due to stimulation of afferent fibers is persistent for a considerable period of time after stimulation has ceased.

Both inhibitory and excitatory effects on other muscles than the gastrocnemius resulted from stimulation of the afferent fibers in the nerve to the gastrocnemius. Stimulation of the larger fibers, alpha and beta groups of the tibial nerve at the level of the knee resulted in inhibition of the gastrocnemius and contraction of the quadriceps extensor. The larger fibers in a muscle nerve then initiate a reflex with a simultaneous double sign. The flexors at the knee have their reflex tone inhibited while the extensors are thrown into a contraction. If the strength of stimulus is made strong enough to excite fibers of the pain fiber size (6 to 3μ) and the

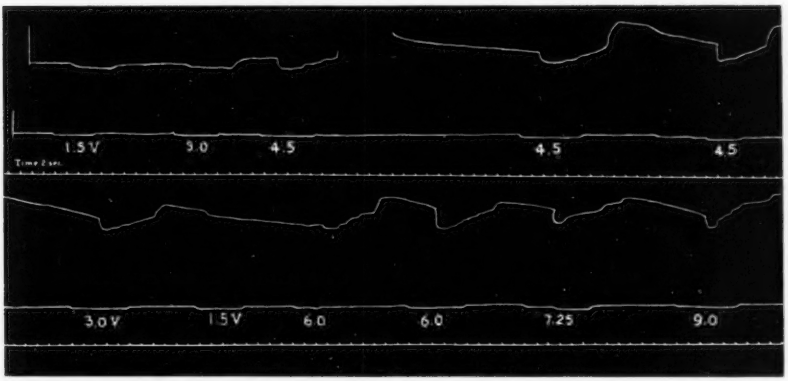

Fig. 15. Myogram of gastrocnemius muscle on stimulating nerve branch to lateral head, branch to medial head intact. Down stroke relaxation, upstroke contraction.

frequency of stimulation is adequate, both muscles are reflexly contracted simultaneously. Our results indicate, then, that coördinated reflex adjustments, phasic or postural, are the result of activity in afferent fibers well above the pain fiber size range; whereas, tonic reflexes result from activity in the smaller afferent fibers. The excitatory effect from these fibers tends to spread beyond the coördinated pathways, so that if the number of such fiber stimuli is adequate, antagonistic muscles may be simultaneously thrown into muscular contraction.

The latter circumstance seems to us to bear on a confusion that exists concerning many experiments on reflex action involving muscles. It is obvious that in a normal intact subject a stimulus, not painful, but of the character of touch or pressure, may cause a coördinated and purposive response; and that a painful, and especially a sudden or violent stimulus

may cause a start or a spasmodic tenseness which is not well coördinated, and which involves the simultaneous contraction of antagonistic muscles in a rigidity which is not immediately purposive. These two types of responses seem to be the counterparts of the two types of responses to be obtained in lightly anesthetized animals, from stimulation of large and of smaller fibers in either skin or muscle nerves. A distinction should thus be observed between responses, inhibitory or the reverse, which involve orderly and effective movements of a coördinated character, by reciprocal mechanisms in the cord, and those more generalized and violent responses which spread beyond the coördinated pathways and result in what is effectively a spasm. This is not to say that nociceptive stimuli do not result in coördinated reflexes, but rather that the reflexes so resulting are liable to be distorted by uncoördinated spread of stimulus so that their analysis is confusing. One of the significant data for an interpretation of reflexes should, therefore, be the strength and character (frequency, etc.) of the stimulus, to correspond to the varieties of fibers that are involved in its initiation, and to the type of afferent effect, (sensation, etc.) that is produced. There is presumably discrimination at the reflex level between different stimuli, which is of the same character, though perhaps not of the same refinement, as sensory discrimination at higher levels.

The responses to the arbitrary stimulation of only the large sensory fibers from muscle are apparently of the nature of proprioceptive postural responses, although the pattern of this stimulation is obviously not the normal one that the muscle would produce. The responses to stronger stimuli, involving pain fibers, if the frequency is not too high, may also be coördinated. We have not examined such responses in sufficient detail and in enough different muscles to analyse them. With high frequency and strong stimulation, antagonistic muscles may both be thrown into tetani approaching the maximal from direct stimulation, but interrupted irregularly to give a semblance of coördination. The reversal of the response of the gastrocnemius on strong stimulation may not, as far as we know, mean that the proprioceptive reflex to nociceptive stimuli is typically a contraction, but rather, may indicate that extreme stimulation results in overflow from the pathway that typically gives inhibition of this muscle, to result in a general spasmodic response that includes the otherwise inhibited muscle itself. At any rate, then, two responses differ in character further than in simple directions of effect.

SUMMARY

1. Fiber counts according to size of the nerve to the median head of the gastrocnemius of the cat, in the normal preparation, after cutting of dorsal roots, of ventral roots, and after sympathectomy, have been plotted against the reciprocal of fiber diameter, and against the diameter. The area of

fibers in osmicated cross sections has been plotted against the reciprocal of the diameter, to compare with action potentials of the same preparations.

2. About equal numbers of afferent and of efferent fibers are present, both in the large fiber range and in the small. The efferent fibers fall into two well-defined groups on either side of 8μ (confirming Eccles and Sherrington). The afferent fibers show no such division when plotted reciprocally, the partial division evident in the curve for the whole nerve is, therefore, due entirely to the efferent groups. About 30 per cent of the myelinated fibers are below 8μ diameter.

3. The non-myelinated fibers in this muscle nerve enter mainly from the dorsal roots, with few from the ventral roots or from the sympathetic. They comprise about a quarter of the total numbers of fibers in the nerve, and a correspondingly larger fraction of the fibers from the dorsal roots.

4. The third cranial nerve, plotted similarly, shows no such divisions and since the ocular muscles have been reported to contain no muscle spindles, it is inferred that the latter are innervated in the nerve to the gastrocnemius by the small-fiber motor group.

5. The potentials of normal and operated roots and nerves correspond reasonably to the histological picture on the basis of relationships between fiber size and potential previously established.

6. The curve of tension developed against increase of stimulus for the gastrocnemius is closely parallel to the curve of area of potential of the nerve stimulated, rather than to that of numbers of nerve fibers stimulated in deafferented nerves; and also parallel to the curve of fiber cross-sectional area against fiber diameter.

7. The effect of stimulating the small-fiber motor group is too small, if any, to be detected in the presence of the effect of stimulating the fibers of lower threshold and larger size.

8. If, as inferred, the large-fiber group is the functional group accounting for muscle contraction, and the small-fiber group innervates muscle spindles, these two functional groups of fibers have dimensions consistent with those of other mixed nerves, where the usual finding is that fibers serving a common function have a range of threshold, conduction rate, and diameter of about 1:3.

9. In spite of the fact that in nerves with motor roots cut, the remaining afferent fibers do not show such marked grouping as is evident in skin nerves, the size range assignable to a common function appears to have the usual limits. The functional groups are so closely confluent and so little different in numbers as to be obscured histologically and physiologically, but are still demonstrable by sensory or reflex tests.

10. Besides pressure and pain, reflex vasomotor and respiratory effects were obtained by afferent stimulation, but the separability of these fibers from sensory fibers is not demonstrated.

11. Stimulation of the large afferent fibers above the range of pain fibers in a branch innervating the gastrocnemius results in release of tonus if this is present in the gastrocnemius; otherwise this has no effect on tension; stimulation of fibers in the size range of pain fibers results in contraction, in lightly anesthetized cats. The same procedures produce reversed effects in the quadriceps extensor, except that if the strength and frequency are sufficient, spasmodic contractions of all the muscles in this vicinity obscure the simple coördination.

12. To analyze adequately reflexes involving muscles and to differentiate between those of postural coördination and those of tonic or spastic character, the functional content of the afferent nerves involved, and the strength and frequency of stimulation as determinants of the afferent effect, are essential data.

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BLOOD AND PLASMA VISCOSITY DETERMINED BY THE METHOD OF CONCENTRIC CYLINDERS

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During the past quarter of a century investigations have shown that the law of flow through a glass capillary, deduced by Poiseuille, does not hold for many colloidal solutions and suspensions. With these liquids, the quantity discharged in unit time is not simply proportional to the pressure but increases more rapidly than the pressure. Likewise, with concentric cylinder viscometers, it has been demonstrated that the moment does not increase proportionally to the angular velocity of the outer cylinder, but less rapidly than this velocity. Hence, for fluids of this nature, the viscosity coefficient is not a constant; the value found depends upon conditions obtaining in the viscosity apparatus.

Hess (1911, 1920), Rothlin (1920) and Rothmann (1914) showed that with blood, and plasma, capillary tube viscometers give high viscosity values if too low pressures are used and consider the driving pressure a pertinent point in the construction of instruments intended to measure the viscosity of blood. With pressures above approximately 200 mm. of mercury these authors found that blood obeys the law of Poiseuille. Fåhraeus and Lindqvist (1931) state that the minimum, or critical, pressure below which the viscosity of blood increases with a decrease in pressure, varies with the length of the glass capillary; also, with the size of the tubes employed by them, a pressure of 100 mm. was far above the critical level. These authors show further that when observations are made with blood, the results depend upon the size of the capillary used; if its diameter is smaller than 0.3 mm., the values decrease below this size with the diameter of the tube.

The results of the investigation to be reported here deal with the viscosity of the blood and of the plasma determined with a concentric cylinder apparatus.

METHOD. The viscometer used by us (fig. 1) is similar in principle to one described by du Noüy (1923) and used by him to determine plasma and serum viscosity. Changes in construction were found necessary, but only essential alterations will be mentioned here.

A thin aluminum cylinder, turning in oil and mounted so that the axis of rotation of the plunger passes through its center, serves as a damping device.

The rotating cup is so constructed that near its upper end there is a larger bore than the one in which the plunger is immersed. This allows a sharp edge from which a convex meniscus of liquid can be formed by filling the narrower part of the cup to slight excess. The lateral pull of the liquid upon the plunger is made equal in all directions, contributing to a stable zero point, and readings practically free from any oscillation. Transparent bakelite was used as the material for the cup and plunger because of its unusual properties with respect to blood as shown by Lampert (1931). The cup has an 8 mm. inside diameter at the narrow bore and its length is 24 mm. including at the bottom a 60° taper. The plunger is 6 mm. in diameter and 14 mm. in length, the lower part also tapered to a 60° angle. It is hollowed out and the space in the center filled with mercury. This adds the necessary weight to insure stability. The cup fits into an aluminum holder so that it can be taken out for cleaning.

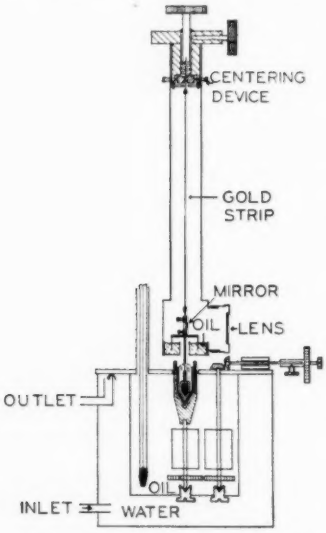

Fig. 1. Cross section of viscometer to show details of construction.

That part of the apparatus into which the cup fits, and which contains the oil and water bath, etc., can be elevated or lowered by turning a large wheel. This part also swings clear of the plunger when in the lowered position, making the latter easily accessible for cleaning. When in place, the clearance between the cup and the plunger is 1 mm.

The torsion wire is a 0.004 inch gold strip obtained from Leeds and Northrup and is approximately 150 mm. long.

A telechron tower clock motor anchored to the apparatus rotates the cup and stirs the oil in the temperature bath. The cup can be rotated at speeds of either 6 or 14 revolutions per minute by the use of two pairs of gears. These speeds are sufficient for determining viscosity up to about forty times that of water.

The correct temperature is maintained by circulating water from a constant temperature bath. The temperature control should be accurate to at least 0.1°C.

To make a determination, the cup is partially filled with the liquid and raised into place; sufficient liquid is then added by means of a slender pipette to produce the elevated meniscus. It is necessary to have blood at about the temperature at which the viscosity is to be determined and well mixed before it is placed in the cup, in order to prevent settling of the cells before a constant reading is obtained.

In using the instrument, the scale readings for water are found at a given temperature. The ratio of the scale reading of the liquid tested to that of water at the same rotation speed, expresses the viscosity of the unknown material relative to water at that temperature.

The instrument is best checked for accuracy with cane sugar solutions of 20, 40 and 60 per cent concentrations by weight as recommended by Bingham and Jackson (1917). The percentage of the solution should be determined by measurement of the specific gravity. The relative viscosity of

TABLE 1

A table showing changes in blood viscosity with various speeds of rotation of the cup

HEMATOCRIT	SPEED OF ROTATION OF CUP (REVOLUTIONS PER MINUTE)							
	4.2	8.2	12.7	19.7	25.0	48.0	75.0	116.0
22.7	6.0	5.3	5.1	5.0				
28.3					3.1	2.9	2.8	2.8
35.1					5.0	4.3	4.1	4.0
38.5	14.9	11.4	10.2	9.1				
44.2					7.6	6.6	6.0	6.0
51.1	28.0	22.3	20.3	18.3				
56.2					23.2	18.8	16.6	14.9
69.5	65.3	48.6	41.6	34.8				

the sugar solution multiplied by the viscosity value for water in centipoises at the same temperature will give the figure to be checked against the accepted results published by these authors. An additional check is to determine and compare the viscosity of the same solution at both rotation speeds of the cup which will show any evidence of turbulent flow at the higher speed. An extended series of determinations with sugar solutions at both rotation speeds and at different temperatures, shows that the instrument described gives results accurate to within 1 per cent.

Blood was obtained by cardiopuncture from cats and rabbits; venipuncture from human subjects with the aid of a lightly applied tourniquet; and from the femoral artery of dogs.

A bakelite syringe was so calibrated that 0.1 cc. of a 10 per cent potassium oxalate solution could be drawn into the syringe, and blood collected rapidly but without bubbling to the 5 cc. mark. The blood was mixed

gently in the syringe, transferred to a paraffin coated test tube and was never in contact with glass surfaces at any time. The potassium oxalate caused a slight shrinkage of the cells besides introducing a 2 per cent dilution factor. Attempts to make determinations without an anticoagulant were not successful for the reason that the scale reading did not remain stationary but moved rather quickly in the direction of increasing viscosity. This occurred even though the total time interval, including withdrawal of blood and taking a reading, did not exceed thirty seconds. No special precautions were taken as to time of obtaining blood in relation to food

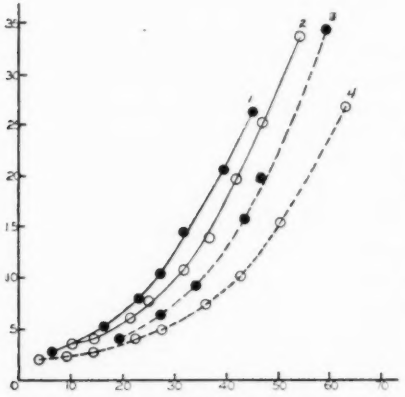

Fig. 2

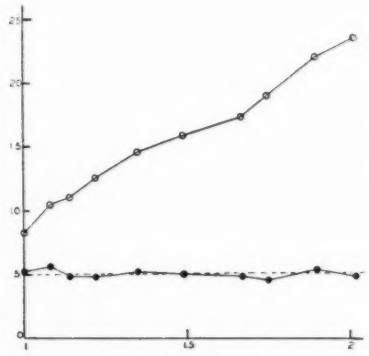

Fig. 3

Fig. 2. Blood viscosity plotted as ordinates against hematocrit as abscissae. 1, cat blood, plasma viscosity 1.84, mean corpuscular volume 37. 2, dog blood, plasma viscosity 1.84, m.c.v. 83. 3, human blood, plasma viscosity 1.66, m.c.v. 91. 4, rabbit blood, plasma viscosity 1.60, m.c.v. 58. Viscosities relative to water at 37°C.

Fig. 3. Blood viscosity plotted as ordinates against plasma viscosity as abscissae. The solid circles represent the hematocrit for each of the blood viscosity determinations and shows small deviations from a hematocrit of 50 indicated by the broken line. Dogs blood. Viscosities relative to water at 37°C.

and water, except with human blood, which was withdrawn after an interval of about four hours following the last meal.

All readings were taken at a temperature of 37°C. With the exception of table 1, all blood viscosity values were determined with a rotating speed of 6 revolutions per minute; plasma values were determined at a speed of 14 r.p.m.

Results. Average values for blood and plasma viscosity respectively were found to be as follows: rabbits (11 animals) 6.6 and 1.68; cats (11 animals) 11.1 and 1.72; dogs (18 animals) 14.6 and 1.77; humans (6 individuals) 13.2 and 1.64. Hematocrit readings were taken of approxi-

mately half the blood samples. These averaged as follows: rabbits 28, cats 33, dogs 44, humans 43.

Figure 2 illustrates the effect of the volume of red cells upon the viscosity of the blood, and shows that the viscosity increases at a faster rate than the hematocrit. For these determinations blood was collected, centrifuged, and the plasma viscosity determined. To the plasma, varying amounts of red cells were added, and the hematocrit and blood viscosity determined for each mixture.

Figure 3 shows the effect of the plasma viscosity upon the blood viscosity. These results were obtained by mixing constant volumes of vari-

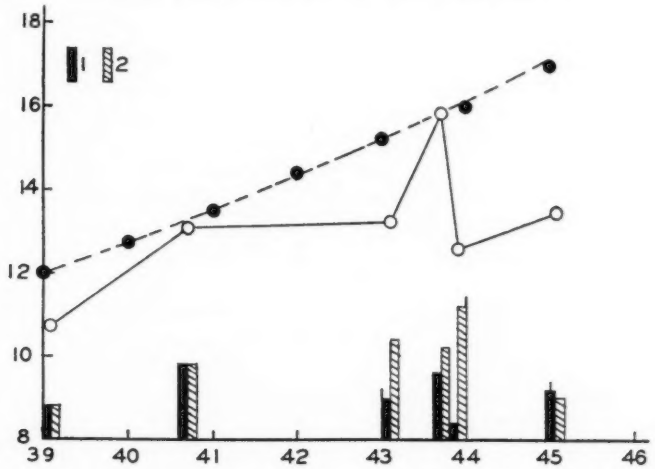

Fig. 4. Blood viscosity (ordinates) plotted against hematocrit (abscissae). Solid circle curve has a constant plasma viscosity of 1.66. Plasma viscosity (solid blocks) and mean corpuscular volume (shaded blocks) are charted for each value of the open circle curve. The base line is equal to a plasma viscosity of 1.58 and a m.c.v. of 80 c. micra; the height of 1 represents 0.04 plasma viscosity above 1.58, and the height of 2, a m.c.v. of 4 c. micra above 80. Human bloods. Viscosities relative to water at 37°C.

ous dilutions of plasma of known viscosity with approximately constant volumes of red corpuscles. The curve confirms the work of Trevan (1918) that the viscosity of blood is a linear function of the plasma viscosity.

In figure 4 are plotted the results obtained with human blood.

It is apparent that the values for blood, obtained at a rotating speed of six revolutions per minute, are considerably higher than the usual values given for blood viscosity. For this reason, the 0.004 gold strip was replaced by one having a higher torsion value, and mechanical arrangements made to rotate the cup at higher speeds. The results of viscosity determinations

of blood at these faster speeds are given in table 1. No observations were made of plasma at high speeds, inasmuch as determinations made at 6 and 14 revolutions per minute give the following values:

Speed 6	1.70	1.70	1.65	1.67	1.85	1.74	1.77
Speed 14	1.72	1.70	1.63	1.67	1.85	1.74	1.80

The decreased value of blood viscosity at higher rotating speeds is not due to an erroneous value for water caused by turbulent flow. Turbulence develops in water only at linear speeds above 4,000 cm. per minute (Couette, 1890a, 1890b) (Mallock, 1896), whereas the maximum linear speed used in our apparatus was 290 cm. per minute; in addition, the scale readings for water were proportional to the rotating speed of the cup.

Discussion. The viscosity of blood is anomalous, since it decreases with increasing angular velocity until a certain velocity is exceeded, above which minimum and constant viscosity values are obtained independent of angular velocity. It is doubtful, however, whether the minimum viscosity value is any more significant, physiologically, than those obtained at lower angular velocities. Investigations by Hatschek (1928) and by Trevan (1918) suggest that the easily deformable character and elasticity of the red cells may be responsible for the variations of viscosity determined at different conditions.

The viscosity of blood is influenced by the volume of red cells and the plasma viscosity; the size of the cell appears not to be a determining factor (figs. 2 and 4).

Our results show that a linear velocity of about 200 to 300 cm. per minute is necessary to obtain minimum values of blood viscosity. The conditions in the larger vessels are such that the blood should obey the law of Poiseuille. Where the velocity is less as in the smaller vessels, the blood viscosity would apparently be higher. On the other hand, the results of Fåhraeus and Lindqvist (1931) indicate, however, that in these smaller vessels, below a diameter of 0.3 mm., the blood viscosity becomes progressively less with a decrease in the diameter of the vessel. They believe this decrease in viscosity is due to a relative decrease in the number of red cells brought about by axial flow. Hence, the effect upon viscosity of velocity changes and the changes in the caliber of the vessels tend to offset one another.

SUMMARY

1. A modified concentric cylinder viscometer for determining viscosity of small quantities of liquids is described.

2. The viscosity of blood as determined by this method is not a constant, but depends, up to a certain point, upon the speed of rotation of the outer cylinder.

3. The viscosity of blood is influenced by the plasma viscosity and by the volume of red cells.

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THE TIME OF OCCURRENCE OF THE CORTICAL RESPONSE AS DETERMINED BY THE AREA OF THE STIMULUS OBJECT[1]

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The relation of intensity and duration of light falling on the retina to the latency of cortical response was reported recently (Bartley, 1934). Under the conditions obtaining, the temporal relations of stimulus to response were quite similar in type to those found for the retina and optic nerve, and this was taken to mean that most of the time elapsing before the appearance of the response was consumed in the retina in getting the impulses under way, only a small fraction of the time being spent in conduction and at the thalamus. The experiments of Bishop (1933) in which cortical response followed electrical stimulation of the optic nerve as closely as 5 sigma, lends support to this assumption.

Our present concern is the function of stimulus area in determining the time of occurrence of the cortical response which, it appears, will include as a major element the time required for the retina to respond, but also a variable latency at the thalamus. The rationale of the effect at the thalamus of area of retinal stimulation appears below.

It has already been demonstrated by various investigators, that area is a factor in determining the latency of the response which they measure. Ishihara (1906), Adrian and Matthews (1927–28), and Granit (1933) have shown that increase in area shortens reaction time. The latter found, for example, that within the range of about 0.7 to 7 degrees, the latency of the action potential of the cat's eye was a linear function of the logarithm of the stimulus area. Adrian and Matthews (1928) analyzed the area effect, attributing it to a neural interaction in the retina, and showed its spatial limitation.

Further analysis of the area factor is possible both by extending the visual angle and by extending the amount of the optic pathway studied. In the present situation we have done both. At times a visual angle of 90 degrees was reached though the greater number of experiments was per-

[1] The work was done under a grant-in-aid for Research in Neurophysiology from the Rockefeller Foundation.

formed with a range from 1.5 to 46 degrees. The upper limit of this range exceeded that used by Adrian and Matthews which in their experiments extended in some cases to the neighborhood of 30 degrees.

METHOD. The rabbit's cortical response[2] to short flashes of light was measured by the cathode-ray oscillograph and the necessary vacuum tube amplification. The animal was lightly anesthetized with ether through a tracheal cannula. It has been shown that immobility can be produced in this way in the rabbit without greatly altering the appearance of the spontaneous cortical activity or obliterating cortical response to optic nerve stimulation (Bartley and Bishop, 1933). Stimulation was produced by one of three different sets of apparatus. The first stimulus series employed the illuminated face of a three inch biconvex lens whose exposed surface was reduced in 8 steps by diaphragms from 2 inches down to $\frac{1}{4}$ inch in diameter. The animal's eye was about 15 inches from the lens. The second series employed a set of 9 apertures any one of which could be used to expose a uniformly lighted field of opal glass. These apertures ranged from 8 inches down to $\frac{5}{16}$ inch. The eye was placed 4 inches from the field. The third series employed 10 apertures reducing the exposed face of a $4\frac{1}{2}$ inch plano-convex lens in steps to $\frac{1}{8}$ inch. The eye in this series was $5\frac{1}{2}$ inches from the lens. The visual angle varied in series I from about 50 minutes to about 7.5 degrees; in series II from 7 to 90 degrees; and in series III, from 1.5 to 46 degrees. The intensities in the three series were respectively 10 to 150,000 c./ft.^{2}; .05 to 525 c./ft.^{2}; 8 to 3175 c./ft.^{2} In each series the necessary short exposures of light (about 40 sigmas) were produced by a set of revolving discs with the appropriate open sectors, which in series I were at the light source, in II at the eye, and in III at a focal point in the optical system. The duration of the flash was long enough for the critical duration to be always exceeded.

The ground electrode was on moist cotton placed on the naked skull in front of the cranial opening while the active lead was on the surface of the brain with the dura removed. With the methods used so far, no point-to-point relationship has been manifest between retinal and cortical areas, although some differences in implicit time have been demonstrated as one proceeds laterad across the cortex. Under the present conditions the active electrode cannot be taken to represent any specific portion of the retina but rather the summed activity of the whole.

RESULTS. The outcome of varying area with constant intensity and duration is shown in figure 1. It will be noted first that increments in area reduce implicit time throughout the entire range used, and there is no reason to believe that this effect would vanish until virtually the whole retina is occupied by the stimulus. The curves are not smooth, however,

[2] Measurements were made from the beginning of stimulation to the peak of the first response potential. The interval has been called the *implicit time*.

as are those obtained by varying either duration or intensity with constant area. Implicit time is reduced very rapidly while the stimulus subtends only a small visual angle, that is, while the retinal image is very small. But as the image is increased, the *rate* of this shortening is reduced con-

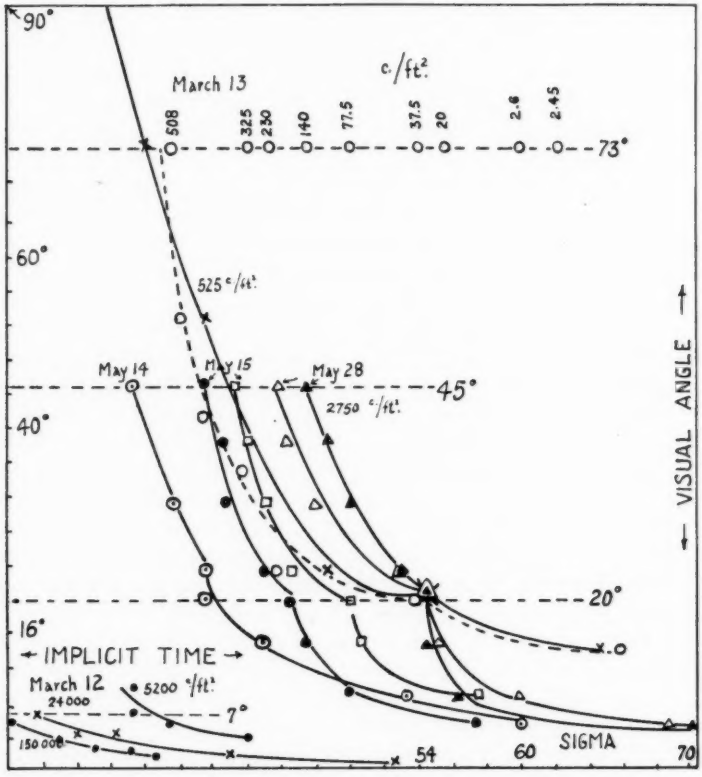

Fig. 1. Curves showing relation between implicit time and stimulus area, and manifesting an abruption in the region of 20 degrees of visual angle. Below this point the curves are formed by the coöperation of an increasing spatial summation within the image and an increasing intensity over the whole retina. Beyond this point the latter alone is increasing.

siderably. The minimum rate is reached when the stimulus subtends a visual angle in the neighborhood of 20 degrees, beyond which it abruptly increases to diminish gradually as the angle is made greater. However, no claim can be made as to the exact point of the abruption. Had our curves not extended any further than the region of the abruption we

might have supposed we had demonstrated an instance of the Adrian and Matthews interaction effect and would have been unaware of any other process in further reducing implicit time, for near the abruption an increase in area scarcely reduced implicit time at all.

But since our experiments showed that implicit time may be reduced still further, we have evidence that retinal interaction alone is not responsible. And it would seem that the conditions in our experiments are representative of the usual situation both in life and in experimentation.

The following suggests what is responsible in addition to retinal interaction for determining our results. Examination of the excised eye under conditions similar to those employed with the living anesthetized animal revealed that a considerable amount of stray light fell on the retina even when the image was sharply focused. A report of this appears elsewhere (Bartley, in press). It was shown in several ways that this general illumination was too great to be ignored.

1. Photometry of the external globe of the excised eye revealed that the ratio between the illumination of the image and the remainder of the retina under a great variety of conditions was indicative of a functional amount of light falling on the retina outside of the image.

2. Cortical responses were elicited by light falling only on the sclera, the pupil having been shielded. Such light upon entering the eye is distributed over the whole retina. Responses from it show its effectiveness as a part of the usual input to the eye.

3. The comparison of the necessary intensity for threshold cortical response for the whole eye evenly illuminated with one having a small image and the attendant stray light, showed the low even illumination and the stray light to be of the same order.

That stray light plays an appreciable rôle in visual phenomena was pointed out by Cobb (1914) in experiments on the effect of bright surroundings on human foveal vision.

If the stimulus area operates in determining implicit time in both of the ways just suggested, namely, by controlling the effective interaction within the image and by determining the level of stimulation over the whole retina, one ought not to expect the curve for stimulus area and for stimulus intensity to coincide throughout. Since interaction and intensity are both varied when stimulus *area* is manipulated and intensity alone when stimulus *intensity* is manipulated, the curve representing the relation of the former to implicit time should lie to the left of the one for the latter. That is, as long as interaction has not reached its maximum effect, increasing stimulus area should shorten implicit time more than increasing intensity, after reaching which the two should coincide. This is precisely what is implied by the shape of our area curves.

A measure of this can be obtained from the two curves in figure 2. The

curve to the left represents the effect of varying area with an intensity of 2400 c./sq. ft. while the other shows the same variations with an intensity of 1900 c./sq. ft. a reduction of about 21 per cent, which reduction (with a stimulus subtending 7.5 degrees), as measured by transferring from the one curve to the other, lengthens implicit time about 2.25 sigma, while a similar reduction in *area* scarcely changes implicit time at all. Starting with a still smaller area (one subtending 3.7 degrees), a 21 per cent reduction in *area* increases implicit time about 1.5 sigma, and an equal reduction in *intensity* increases it about 3.75 sigma, showing that the intensity curve when constructed would fall to the right of the area curve with which it coincides at the top, in keeping with the expectation just outlined.

It thus appears that our curves in figure 1 represent the composite effect of the increase in intensity of stray light scattered over the *whole retina* and the increase in the effective spatial summation within the *image* as stimulus area is enlarged, the latter effect reaching its limit long before the whole eye is occupied by the enlarging image. The point at which this interaction halts is not essentially different than that expected from Adrian and Matthews' findings.

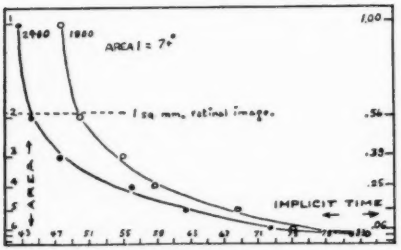

Fig. 2. Area vs. implicit time, one curve with intensity 21 per cent less than the other. By comparing a 21 per cent area reduction with it, one can see the discrepancy between manipulating stimulus area and intensity while the retinal image is small. Implicit time in sigmas, area in relative terms. The largest, a circle subtended by a visual angle of 7.5°.

A check on this assumption is to be had by comparing an increasing intensity of uniform illumination over the entire retina with an increasing intensity upon a retina provided with an image and the consequent stray light. Such a procedure would compare the effect of intensity as a factor acting on the whole retina with the function of a given image along with this illumination in determining implicit time. We have an example of this in figure 3, although not over a very great intensity range. The left-hand curve represents the kind of distribution found in figure 1, except that intensity instead of area is varied. Interaction within the image is kept constant by constant image size, and so cannot modify the curve by being a changing factor. The right-hand curve gives the outcome when the image is dispensed with. To be more specific, the left-hand curve shows the result of using a stimulus subtending a visual angle of about 7.5 degrees. The ordinate of the graph is graduated in units of 100 c./sq. ft., to apply to this curve. The intensities of the uniform illumination plotted in the other curve are a constant fraction of those in the first, namely, 0.0022,

and were the same order as the stray light accompanying the image in the first case, as calculated from photometric measurements of the external globe.

It will be seen that the curves are smooth and are not far from being coincident, the addition of the light of the image in the one case reducing implicit time a little, that is, if the image were ineffective and only the stray light stimulated, the curve of implicit time would be moved only 3 sigma out of say 60. The curves may converge at the bottom

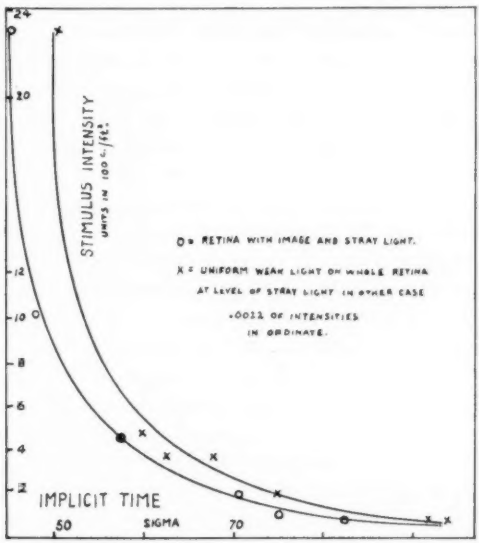

Fig. 3. Curves showing relation of stimulus intensity to implicit time. Left-hand curve represents the retina provided with an image (7.5° visual angle), and the usual stray light. The other curve represents a uniform illumination of the retina equal to the stray light alone.

in keeping with the previous observations that at threshold for cortical response the stray light accompanying a small image must be of the same intensity as an evenly illuminated total eye, the existence of the small image having no measurable effect on the size of cortical response.[3]

[3] The use of an external stimulus producing a very small retinal image even though very bright does not produce a recordable cortical response, it would seem due to the lack of enough stray light to produce the necessary stimulation of the retina outside the image. On this basis, if an image alone (with no stray light) could be produced on the retina one might not expect to produce a recordable cortical response unless the image were large. Though this at first might seem strange, there is an understandable basis for it, namely, that with only a very small amount of tissue active in the cortex such as, for example, is produced by a tiny image, the shunting effect of the remaining mass precludes its being recorded (Bartley and Bishop, 1933).

This test by showing that implicit time is shorter when the image is added to the general illumination and by the otherwise parallel course of the two curves, supports the conclusion given for the area curves, namely, that they represent the sum of the effects of an expanding image and of increasing intensity due to scattering light over the rest of the retina.

Discussion. Our results indicate that the time required for response at a given region in the cortex to a small area of retinal stimulation is shortened by the addition of the effect of the stray light which is scattered over the retina in general. The question is now where the facilitation takes place and whether it is spatial or temporal. The course of events is inferred to be as follows. The impulse from the small area intensely stimulated (image I) reaches the thalamus ahead of the impulse from the rest of the retina (R) but meets with delay there. The R impulse is facilitated in passing the thalamus by the previous activity there of the I impulse. Cortical response to both is therefore approximately coincidental. The facilitation is thus temporal in the sense that impulses starting from the retina at different times due to different intensities of light falling on different areas act together at the thalamus in shortening latency; and spatial in the sense that these impulses travel in parallel but separate fibres. The reasons for these inferences are as follows.

In the first place it has been shown that the image area was not the only part of the retina stimulated. The very first part of the response at the cortex must then represent the *unitary* coöperation of the impulses from the two retinal areas or else the impression from one of them alone. The fact that the retinal area I intensely stimulated will set up impulses ahead of the area R weakly stimulated and so from the outset make for asynchronization, would by itself lead to the conclusion that the onset of the response at the cortex signalled the arrival of impulses from I only. However, our curves are such as to indicate the coöperation of area I and R, assuming the spatial interaction found by Adrian and Matthews to operate.

Both retinograms and records of the optic nerve discharge fail to disclose anything about the synchronization process which would put the impulses from the two retinal areas in tune. If such a process does occur in the retina it must take place where for any reason latency is manipulated, namely, in the outer plexiform layer, for the interval between the discharge of inner plexiform layer signalled by the onset of the retinogram, and the outer plexiform layer signalled by the optic nerve discharge is supposed to be constant.

The next point for which records are available is the cortex. The response here is not similar in its shape to that of the retina or nerve. The development of potential is more abrupt, suggesting that some sort of rearrangement has taken place at the thalamus. It is inferred that the processes from the two retinal areas are synchronized here, because of all

points considered, the thalamus has been the one most clearly shown to provide for interaction and so change temporally the outcome at the cortex. Bishop did this when he demonstrated that parallel paths here exert a mutual influence when by the use of electrical shocks to the optic nerve, (retina removed) he varied the latency of the cortical response through a range of 45 sigma,[4] that is, by simply varying the number of nerve fibers activated. There seems to be ample ground, employing already known facts, for assuming that the two areas of the retina coöperate in timing the unitary cortical response, and for taking the results of our experiments as a demonstration of the temporal outcome. That is, when two adjacent stimuli of different intensities are applied to the retina, and the two responses arrive at the thalamus successively, the second visual impulse will tend to be facilitated by the first, and the interval between them thus decreased or obliterated, the first impulse having a longer latency at the thalamus than the second.

A word needs to be added about the relation between the stray light on the retina and the experience resulting from it. Upon first thought, there might be some doubt as to the impulses from the retinal periphery ever reaching either the thalamus or cortex, for it is usual to think of the stimulus affecting the retina only at the image site and thereabouts, and not to cause the animal to see the whole visual field lighted up. At least it is known that object localization and discrimination between points exist. However, if a small bright spot in a dark field is intermittently illuminated (made to flicker) the whole dark field may be seen to flicker also. This indicates that the field as a whole *is* intermittently illuminated by the small image to an amount productive of sensation. Without this observation, that is, under ordinary conditions, the peripheral field is called black due as much to the relativity of what is really black and to the known crudeness in making comparative brightness judgments except when two areas are being equated, as to the weakness of stimulation.

SUMMARY

1. The area of the external stimulus was varied through a range of 1 to 90 degrees in visual angle, throughout which range increments in area reduced implicit time of cortical response.

2. The relation between stimulus area and implicit time is not a simple function similar to that of stimulus intensity.

3. Its irregularity is due to the fact that the image is not alone the site of stimulation. The remainder of the retina receives a material amount of stray light, so that curves depicting the relation between stimulus area and implicit time are composites of the increasing spatial summation within the image and the increasing intensity on the rest of the retina.

[4] A ten-fold change from minimum to maximum latencies.

4. The matter of synchronization in the two portions of the retina and of mutual facilitation at the thalamus in producing a unitary cortical response are discussed.

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THE NATURE OF VENTRICULAR FIBRILLATION FOLLOWING ELECTRIC SHOCK AND ITS PREVENTION BY ACETYL-β-METHYL CHOLINE CHLORIDE

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Electrocardiographic studies (Hooker, Kouwenhoven and Langworthy, 1933) of the effect of electric shock of low voltages administered through the body of experimental animals show clearly that death is due primarily to ventricular fibrillation. These studies suggest strongly that fatalities in accidental shock from household fixtures and other sources of low voltage are probably also of cardiac origin. Since short shocks of low voltage seem to produce few irreversible changes in the central nervous system (Langworthy, 1930), measures designed to reëstablish and maintain effective cardiac contraction may be expected to prevent a fatal outcome. It becomes important, therefore, to understand more fully the physiology of ventricular fibrillation in such circumstances.

In recent communications (Nahum and Hoff, 1934; Hoff and Nahum, 1934) we have demonstrated that ventricular fibrillation may result from the action of adrenaline on a ventricular myocardium that has been previously sensitized by some agent such as benzol. In all such cases the appearance of ventricular rhythms could be prevented by the injection of adequate doses of acetyl-β-methyl choline chloride, which antagonized the action of adrenaline in the body. It seemed appropriate therefore to determine whether acetyl-β-methyl choline chloride would prevent ventricular fibrillation usually produced by electric shock, and whether adrenaline or "sympathin" plays a part in the genesis of the ventricular fibrillation from this cause.

METHODS. Cats were anesthetized with sodium amytal, and tracheal cannulae inserted for artificial respiration. A double throw switch permitted electrocardiographic registration from lead II and the administration of electric shocks from a 110 volt A.C. outlet through the same leads. This arrangement prevented damage to the recording instrument yet permitted registration immediately following the shock.

RESULTS. A. *The prevention of ventricular fibrillation by acetyl-β-methyl choline chloride.* In every experiment (10) a shock longer than 0.5 second resulted in immediate ventricular fibrillation of high frequency (600–800

per min.). Although individual preparations varied, a shock of 2 seconds' duration generally resulted in ventricular fibrillation for 15 to 30 seconds followed by resumption of the normal rhythm (fig. 1). In such animals, respiration stopped and artificial respiration was required for a few minutes, after which spontaneous respiration was reëstablished. A shock of 3

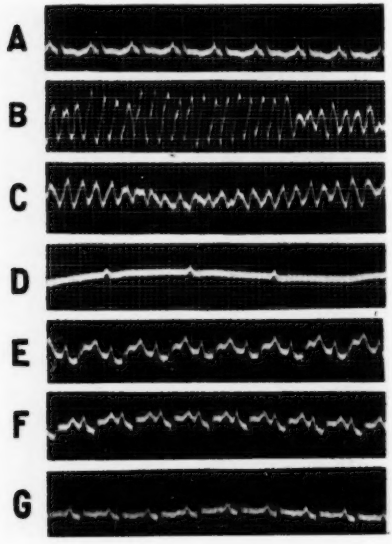

Fig. 1

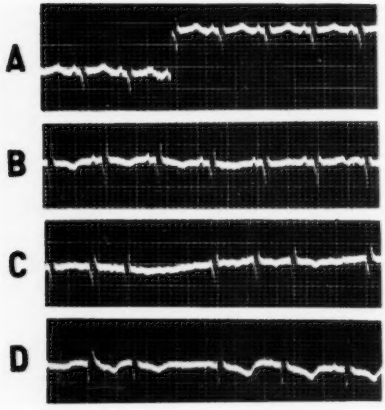

Fig. 2

Fig. 1. Experiment 121, July 12, 1934. Cat. 2.0 kgm. Sodium amytal anesthesia. A. Control 10:00 a.m. B. Ventricular fibrillation immediately after a shock of 2 seconds, 110 v. A.C. 10:10 a.m. C. Ventricular fibrillation 45 seconds after B. D. Ventricular standstill 15 seconds after C with a few auricular complexes. E. Return of normal rhythm, with anoxemic ventricular complexes, after 30 seconds' cardiac massage with artificial respiration, 10:11½ a.m. F. Reduction of anoxemic symptoms 10:14 a.m. G. Practically normal complexes 10:20 a.m.

Fig. 2. Experiment 119. July 9, 1934. Cat. 2.6 kgm. Sodium amytal anesthesia. A. Control 11:00 a.m. B. Ten minutes after subcutaneous injection of 25 mgm. acetyl-β-methyl choline chloride 11:12 a.m. C. Immediately after shock for 3 seconds, 110 v. A.C.; auricular fibrillation. D. Resumption of normal rhythm 1:45 p.m., showing the transition from auricular fibrillation to the normal mechanism. A control shock of 3 seconds half an hour before A produced ventricular fibrillation for 30 seconds.

seconds almost without exception caused ventricular fibrillation of at least 60 seconds' duration, and resulted in cardiac death. In two instances massage of the heart for several minutes through the relaxed thorax combined with artificial respiration, was able to restore a heart beat and led to complete recovery.

Control experiments with repeated short shocks showed that after an interval of half an hour a second shock did not seem to be more serious than the first, but at shorter intervals a second shock might prove fatal.

In five experiments, 25 mgm. acetyl-β-methyl choline chloride in aqueous solution were injected subcutaneously within 10 to 20 minutes after recovery from the ventricular fibrillation resulting from a 2 to 3 second shock, and after a further interval of 10 to 20 minutes a second electric shock of a duration similar to the first was administered. In all but one experiment ventricular fibrillation did not occur, the heart maintained an expulsile beat and respiration continued without interruption. In the one exception, the second shock was of 4 seconds' duration instead of 2 seconds, and was followed by ventricular fibrillation for 2 seconds. With shocks of longer duration (5–8 sec.) ventricular fibrillation could be produced. Whether larger doses of acetyl-β-methyl choline chloride would protect against these shocks was not determined.

In every instance in which an electric shock was administered in the presence of acetyl-β-methyl choline chloride, although ventricular fibrillation was prevented, *auricular* fibrillation appeared, and was recognized by the absence of P waves in the electrocardiogram and the characteristic irregularly spaced ventricular complexes (fig. 2). The rate, however, was approximately that before the shock, and cardiac output was maintained. As the major effects of the acetyl-β-methyl choline chloride wore off about two hours after injection, a normal rhythm was reëstablished (fig. 2, D).

B. *Adrenaline.* In four further experiments the effect of electric shock following elimination of the major sources of adrenaline and sympathin was investigated. After preliminary controls demonstrated that ventricular fibrillation could readily be produced by short electric shocks, the stellate ganglia and adrenal glands of both sides were removed, thus effectively suppressing the greater part of the cardio-accelerator impulses, and eliminating the major source of adrenaline.

Four hours after this procedure, the animals were again subjected to a shock of somewhat longer duration (3–5 sec.) than that which earlier produced ventricular fibrillation. In every experiment these shocks were now ineffective although the outward signs of shock (convulsions, opisthotonus, etc.) were the same (fig. 3). In one animal in which a preliminary control shock of 2 seconds' duration gave rise to ventricular fibrillation for over a minute, requiring cardiac massage for resuscitation, a shock of 4 seconds was ineffective and a shock of 5 seconds produced fibrillation for only a few seconds.

These animals were then allowed to recover for a half-hour or more and then were given 1 to 2 cc. of adrenaline subcutaneously. Two minutes later they were shocked for 2 to 3 seconds, and in every instance they developed ventricular fibrillation from which two did not recover.

To determine whether adrenaline promotes ventricular fibrillation in the normal animal, two animals were chosen which did not show fibrillation of the ventricles after a shock of 3 seconds, probably because of an excess of the anesthetic (Meek and Seevers, 1934). These received 1 cc. of adrenaline subcutaneously and were subjected to a shock of 2 seconds' duration. Fatal ventricular fibrillation resulted (fig. 4).

Discussion. In these investigations the rôle played by adrenaline in the genesis of ectopic ventricular rhythms is further illustrated, and another

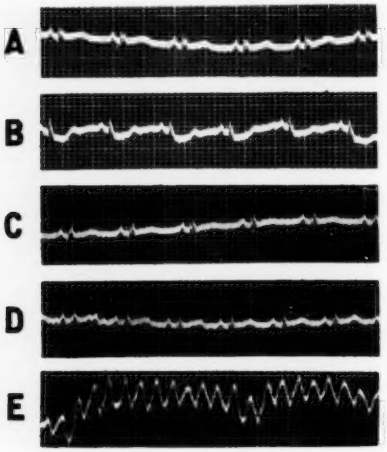

Fig. 3

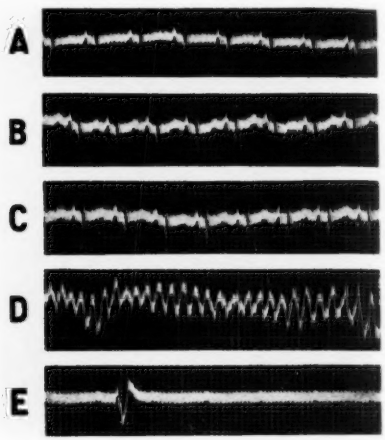

Fig. 4

Fig. 3. Experiment 122. July 16, 1934. Cat. 3 kgm. Sodium amytal anesthesia. Both stellate ganglia and both adrenal glands removed by 12:00 noon. A. Control 4:00 p.m. B. Immediately after shock for 3 seconds. Normal rhythm, with evidences of anoxemia. C. Control 4:30 p.m. D. Two minutes after subcutaneous injection of 2 cc. adrenaline. 4:50 p.m. E. Immediately following 1½ seconds shock at 4:51 p.m.

Fig. 4. Experiment 126. July 27, 1934. Cat. 2.5 kgm. Sodium amytal anesthesia. A. Control 2:30 p.m. B. Immediately following 3 seconds' shock 2:31 p.m. C. Two minutes after subcutaneous injection of 1 cc. adrenaline 3:05 p.m. D. Ventricular fibrillation after shock of 2½ seconds at 3:06 p.m. E. Cardiac standstill.

link added to the chain of evidence that suggests that in the production of ventricular rhythms in general, adrenaline acts synergically with some other factor to raise the rhythmicity of a ventricular focus or foci to such a level that this focus becomes the pacemaker and a ventricular rhythm appears (Hoff and Nahum, 1934). There is a difference, however, between the action of electric shock and that of other agents such as benzol. Dautre-

bande (1933) as well as Nahum and Hoff (1934) found that in acute benzol poisoning ventricular fibrillation usually occurred only in the presence of more than a normal amount of adrenaline in the blood stream, while electric shock is capable of producing ventricular fibrillation in normal cats, and if long enough continued, in animals with the major part or even all of their adrenaline-sympathin apparatus inactive. It would seem, therefore, that the two factors involved in ventricular fibrillation, adrenaline, and an accessory exciting agent, are capable of reciprocal variations. Thus, the greater the concentration of adrenaline in the ventricular tissue, the less need be the concentration of the second factor in order to produce a fatal ventricular fibrillation. Conversely, the more intense the accessory stimulation is, the lower need be the concentration of adrenaline. Thus an electric shock can produce ventricular fibrillation in the absence of even small concentrations of adrenaline. Yet even with the electric shock, removal of a large part of the adrenaline of the body enormously reduces the threshold for ventricular fibrillation, and injection of additional adrenaline greatly enhances the danger of a shock.

Following non-fatal shock, procedures that would increase the concentration of adrenaline in the body such as physical activity or emotional excitement would thus be expected to promote the development of ventricular fibrillation and sudden death. Such cases have in fact been known to occur, where workmen, partially stunned by an electric shock, and resuscitated, have dropped dead on rising and attempting to walk.

The preliminary injection of acetyl-β-methyl choline chloride prevented the appearance of ventricular rhythms following electric shock just as it prevented their appearance after benzol (Nahum and Hoff, 1934) or chloroform inhalation (Hoff and Nahum, 1934). By greatly prolonging the time of exposure to the current it was finally possible to produce ventricular fibrillation despite the dosages employed, but increase of the quantity of the drug administered would be expected to protect against even more prolonged shocks.

The *auricular* fibrillation invariably produced by an electric shock administered in the presence of acetyl-β-methyl choline chloride will be discussed in a later communication.

SUMMARY

1. Ventricular fibrillation in the cat, produced by electric current (110 v. A.C.) applied through electrodes on the right foreleg and left hind leg, has been studied.

2. While a shock of 3 seconds' duration will usually produce ventricular fibrillation lasting for more than a minute and, but for artificial respiration and cardiac massage, would lead to death, such a shock after subcutaneous injection of 25 mgm. of acetyl-β-methyl choline chloride is not followed by

ventricular fibrillation. Effective beats of the heart are maintained, and respiration does not fail. Respiratory failure in such shocks in normal animals must therefore be secondary to circulatory failure.

3. While after acetyl-β-methyl choline chloride ventricular fibrillation does not result from a shock, *auricular* fibrillation invariably does.

4. Removal of stellate ganglia and adrenal glands enormously decreases the susceptibility of the heart to ventricular fibrillation following electric shock.

5. Subcutaneous injection of adequate amounts of adrenaline restores the normal response to shock, and if greater quantities are injected in these or in normal animals, the danger of fatal ventricular fibrillation is greatly increased.

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SOME INFLUENCES OF OESTRIN ON THE HYPOPHYSEAL-GONAD COMPLEX OF THE IMMATURE FEMALE RAT[1]

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Numerous investigations, all ably reviewed by Allen (1932), have established the influence of oestrin on the extra-gonadal female reproductive tract, but the effects of oestrin on the ovary itself have not been so thoroughly investigated. Golding and Ramirez (1928); Spencer, Gustavson and D'Amour (1932); Leonard, Meyer and Hisaw (1930 and 1931); D'Amour (1932); Pincus and Wertheson (1933); Bialet-Laprida (1933) and others have reported an inhibition of ovarian growth and development after treament with various dosages of oestrin. Leonard, Meyer and Hisaw (1931) and Leiby (1933) report an increase in the weight of the pituitary after oestrin injections. Spencer, D'Amour and Gustavson (1932) and Swezy (1933) report an inhibition of spermatogenesis and ovogenesis following treatment with oestrin. Leonard, Meyer and Hisaw attribute the inhibition of ovarian growth and development to an experimentally demonstrable decrease in the gonad-stimulating potency of the anterior pituitaries of the injected animals.

With the development of what appears to be a truly quantitative method of measuring the results of hormone administration upon the ovaries of the rat (Lane, 1935), the present author was interested to determine the effect of oestrin on the follicular apparatus of the ovary of the immature rat; and the possible effects on the gonadotropic hormone content of the hypophyses of the injected animals.

PROCEDURE AND RESULTS. Twenty-two day old female rats were injected with 6.25 R. U. of carefully standardized oestrin[2] per day for periods ranging from 5 to 39 days. Their ovaries were studied in the manner described in a previous paper (Lane, 1935). Their pituitaries were implanted intramuscularly into normal twenty-two day old females, only one pituitary being used for each recipient. After five days the recipient was killed and her ovaries similarly studied. Untreated controls of the

[1] Aided by a grant from the National Research Council, Committee on Problems of Sex, administered by Prof. F. L. Hisaw.

[2] The oestrin preparation used in these experiments was Amniotin, kindly furnished by E. R. Squibb and Son.

same age were killed simultaneously with the oestrin-injected rats; the ovaries of the controls were studied and their pituitaries also implanted into normal twenty-two day old females.

The results of this study fall naturally into two groups; the first includes follicle counts of the ovaries from 29 oestrin-injected rats and 22 uninjected controls, the second consists of similar counts of the ovaries from 17 experimental pituitary recipients and 17 control pituitary recipients.

After five days' injection with oestrin (figs. 1 and 2) the ovaries exhibit a total follicle count which is, on the average, 26.8 per cent greater than is found in normal controls. The ovaries of the oestrin-injected rats, in addition to containing more follicles than did the controls, showed 50 per cent more vesicular follicles than the ovaries of normal rats. After ten days of injection the ovaries contained 13 per cent fewer follicles than the ovaries of the untreated controls, but the vesicular percentage was still 40 per cent higher than that found in normal rats of the same age. After 16 days of injection the total follicle count was depressed 41 per cent below the normal, while the vesicular percentage remained consistently higher than the normal. This disparity persisted until, after 39 days of injection, when the controls had all experienced their first ovulation, the percentage of vesicular follicles in the ovaries of the normal and the experimental groups were almost identical. The oestrin-injected rats continued to have fewer follicles in their ovaries than did the controls.

Injection of oestrin in the amounts used in this study first results in an increase in the total number of follicles in the ovary, and then a depression of this number considerably below that for normal animals of the same age. Coincident with this decrease in total numbers of follicles, which is probably due to a decreased rate of formation, as well as follicular atresia, there is a considerable increase in the percentage of vesicular follicles. This increase persists until the time of ovulation, when the percentage of vesicular follicles in the ovaries of the two groups tends to converge.

Implantation of the pituitaries from the oestrin-injected and the control animals suggests that the ovarian effect which has been discussed is probably mediated by way of the pituitary. In the ovaries of the rats receiving pituitaries from oestrin-injected rats (fig. 3) the vesicular percentage of the total follicles was consistently higher than was found in the ovaries of control recipients. The total follicle content of the ovaries of the pituitary recipients (fig. 4) shows that the glands from the oestrin-injected rats were more efficient in raising the total follicle count than were the pituitaries from control animals, up to the sixteenth day of treatment. After this time the pituitaries from the controls were superior to the glands from the oestrin-injected animals as judged by their ability to produce follicles in the recipients' ovaries.

Discussion. It has been shown (Lane, 1935) that the percentage of

vesicular follicles in the ovary is probably a function of either the luteinizing hormone as such, or something closely associated with it, both chemically and physiologically. It was also pointed out that the follicle stimulating

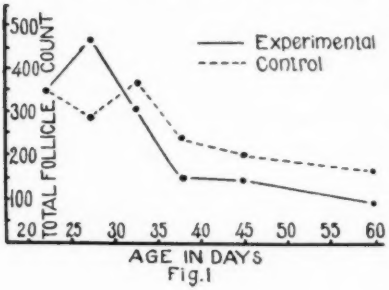

Fig. 1

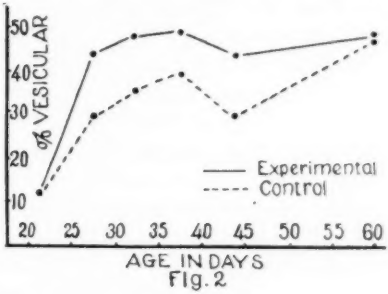

Fig. 2

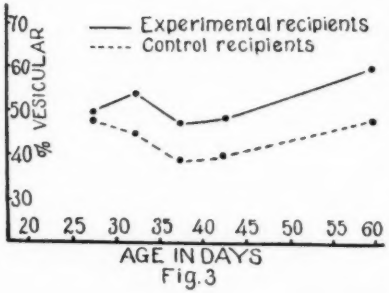

Fig. 3

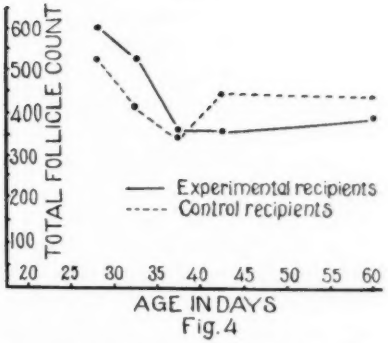

Fig. 4

Fig. 1. The total follicles in the ovaries of oestrin-injected and control rats. The variations in the injected group were so great that the differences observed were not statistically significant until the experimental animals had been injected for sixteen days. After that the differences between the experimental and control counts was always greater than three times the probable error of the mean.

Fig. 2. The percentage of the total follicles which was vesicular in the ovaries of the experimental and the control rats. The differences observed were all statistically significant until the controls had ovulated (61 days). After this time the differences are no longer significant. This would indicate that after ovulation, there was almost as much oestrin in circulation in the control animals as had been injected daily into the experimental group.

Fig. 3. The percentage of the total follicles which was vesicular in the ovaries of experimental and control pituitary recipients. The differences between the experimental and control recipients—after five days of injection—was always greater than three times the probable error of the mean.

Fig. 4. The total follicles in the ovaries of experimental and control pituitary recipients. The differences observed between the means of the two groups, while they are considerable, are not statistically significant due to the great variation in the total follicle counts.

hormone is probably causally related to the numbers of follicles that the ovary contains.

Injection of oestrin, since it caused at first a marked increase in the total number of follicles contained in the ovary of the treated animal (fig. 1), probably stimulated the hypophysis to secrete more of the follicle stimulating hormone than is secreted by the normal hypophysis. This is borne out by implantation experiments in which pituitaries from animals treated for five days with oestrin have a greater follicle-elaborating ability (fig. 4) than have the glands from normal controls when both are implanted into normal twenty-two day old rats. However, after this first period of stimulation has passed, there follows an inhibition of the formation and liberation of the follicle stimulating hormone. This is shown both by the decrease in total numbers of follicles in the ovaries of the injected rats (fig. 1) and by the decreased ability of their pituitaries to produce follicles in the ovaries of recipient rats (fig. 4).

Furthermore, if the luteinizing hormone is responsible for the creation of vesicular follicles, it is apparent that the injection of oestrin stimulates the hypophysis to elaborate and secrete more of this substance than is formed in a normal animal of the same age (figs. 2 and 3). This enhanced formation and secretion was apparently constant for the duration of the experiment. This observation affords confirmation for the findings of Hohlweg (1934) who was able to produce excessive luteinization in the ovaries of adult rats by injection of massive doses of oestrin.

Comparison of figures 1 and 4 will bring out another interesting fact. It will be noticed that the total follicle count of the injected animals (fig. 1) is greater than that of the controls up to 10 days after the beginning of the injections. In figure 4 it can be seen that the hypophyses of the injected rats retain their greater follicle-elaborating ability up to 16 days after the beginning of the injections, whereas this ability could be demonstrated in the ovaries of the owners of these hypophyses for only 10 days.

In figures 1 and 2 it may be noticed that, while oestrin undoubtedly modified the ovaries of the injected rats, this modification was generally but an exaggeration of what normally occurs. The percentage of vesicular follicles in the ovaries of the injected animals reached a level, soon after the initiation of the injections, which was only attained by the normal rats with the onset of sexual maturity. The same factors apparently were operating in both groups, but the injected animals received more oestrin than the normal immature ovary produced until ready for the first ovulation. Similarly with the total follicle count; after the period of stimulation the curve of the total follicle count of the ovaries of the injected rats is simply an exaggeration of the changes established for normal ovaries (Lane, 1935).

The importance of these observations for a rational explanation of the

adult oestrous cycle is quite obvious. Apparently the concept (Moore and Price, 1932) that oestrin has an inhibitory influence on the formation and liberation of the gonadotropic hormones of the hypophysis is only partially complete, overlooking as it does the more important qualitative result of treatment—the stimulation of the hypophysis to secrete increased amounts of the luteinizing hormone.

SUMMARY AND CONCLUSIONS

Six and one-quarter rat units of oestrin per day were injected into twenty-two day old female rats for periods ranging from 5 to 39 days, and the follicle contents of their ovaries counted. The pituitaries of the oestrin-injected rats were then implanted into normal twenty-two day old rats and the recipient's ovaries also studied. From an analysis of the counts the following conclusions may be drawn: Injection of oestrin at first stimulates the hypophysis to liberate gonadotropic hormones in excess of those produced by normal animals. There is then an inhibition of the liberation of the follicle-stimulating hormone and there is apparently a failure of formation of this hormone a few days later. Oestrin causes a greatly enhanced formation and secretion of either the luteinizing hormone from the hypophysis, or of something associated with it, as seen in extracts, which acts in a similar way upon the ovary.

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ON THE INHIBITION OF GASTRIC SECRETION BY OIL OF PEPPERMINT

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In a previous communication (1) we have described the inhibitory influence of oil of peppermint upon human gastric acid secretion, and have reviewed the available and pertinent literature. In our experiments the subjects were peptic ulcer patients, in whom the acid secretion of the resting stomach was either suppressed or diminished by small doses of oil of peppermint administered by stomach tube. Significantly, the acid secretion of the stomach stimulated by alcohol or histamine was also either partially or completely inhibited.

In addition, it was found that human and canine gastric motility, as registered by stomach balloons, remained uninfluenced by oil of peppermint. Yet subsequent fluoroscopy of normal human subjects, the details of which will soon be published, reveals a considerable shortening of the emptying time of a barium meal to which small quantities of oil of peppermint were added.

In an endeavor to further investigate the physiological properties of oil of peppermint, additional research was instituted upon several dogs with Heidenhain and Pavlov pouches; one dog with a gastric and a duodenal cannula, and one bearing a Pavlov pouch and a jejunal cannula.

METHODS. The drugs utilized were oil of peppermint, U.S.P. and histamine acid phosphate. The routine experimental procedure consisted in the collection of at least four ten-minute specimens of stomach or pouch secretion as a control period, then laying the animal supine and introducing into the pouch or stomach either oil of peppermint or olive oil. Five minutes later the dog was again placed erect, and now histamine was injected subcutaneously. When the oil had drained for five minutes, the sampling bottle was re-attached to the cannula (Dragstedt's device), and specimens were collected at ten minute intervals until the volume of secretion had returned approximately to that of the control period. Occasionally, the oils were introduced into the stomach proper through a gastric tube. In the experiments employing dogs with intestinal cannulas rubber

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tubing, 40 cm. in length, was passed through the cannula into the caudal portion of the jejunum, through which the drug injections were made, and collections of gastric secretion were obtained from the pouch or stomach cannula (Kestner cannulas (2)).

In the tabulation of results, volume and acidity (free and combined) of the gastric secretion were plotted against time, and the graphic areas measured with a planimeter. The area of histamine effect was compared with the area of effect with histamine and oil of peppermint, and the relationship expressed as percentage changes in the areas of secretion (as degrees of free acidity in dog 5). Only changes in secretion greater than one-third of the control values were considered significant.

Of course, the various determinations upon the same dog were not performed during the same day, but the time interval between them is brief and presumably unimportant.

RESULTS. *The effect of oil of peppermint instillations into the pouch upon pouch acid secretion:* A. *Oil of peppermint in pouch, secretion of pouch.* Dog 1. Heidenhain pouch. This dog yielded a rather consistent pouch secretion to histamine stimulation. Subsequently, oil of peppermint, in two instances (expts. a and c) completely inhibited free acid secretion from the pouch; and on one occasion (expt. b) induced slight depression. The combined acidity was not elevated, so that the drop in free acidity cannot have been due to an increased production of buffers.

Dog 2. Pavlov pouch. In experiment a, oil of peppermint did not counteract the acid stimulation of histamine. Probably the secretory stimulus of 0.5 mgm. of histamine was too great because, when the dosage was halved, oil of peppermint markedly diminished the acid secretion in 2 out of 3 tests performed (expts. b and c). Unlike the response of dog 1, these 3 experiments revealed a rise in combined acidity which may explain the diminution in free acidity. Yet when this dog was fed his regular diet and immediately afterwards had his pouch filled with oil of peppermint for five minutes, both free and combined acidity of the pouch secretion were diminished appreciably within 30 minutes (expt. e). The free acid dropped from sixty to zero and the combined acid from 90 to 20 degrees. The drop in free acidity, obviously contrary to other results obtained in this dog, is certainly not due to an increase of buffers in the pouch secretion.

Dog 3. Pavlov pouch. Oil of peppermint in the pouch slightly decreased volume as well as free and combined acidity of a pouch secretion stimulated by 1 mgm. of histamine (expt. a), and more or less completely suppressed free and combined acidity after a weaker stimulus from 0.5 mgm. of histamine (expts. b and c). Instillation of oil of peppermint into the pouch, thirty minutes after injection of histamine, still produced a diminution of free acidity without an increase of combined acidity (expt. d).

Dog 4. Pavlov pouch. This pouch was somewhat refractory, respond-

ing weakly to histamine. Oil of peppermint in the pouch promptly brought the free acid to zero, but a slight increase in combined acidity may account for this decrease.

Dog 5. Gastrostomy. Histamine evoked a definite acid secretion from the stomach. Instillation into the stomach of 5 cc. (expt. b) and 30 cc. (expt. a) of oil of peppermint completely suppressed gastric secretory response to histamine.

B. *The influence of oil of peppermint in the jejunum upon gastric acid secretion.* Dog 1. Jejunal cannula and Heidenhain pouch, dog 5. Gastrostomy and duodenal cannula. The presence of oil of peppermint in the jejunum did not stimulate the stomach (dog. 5, expts. a–d), nor did it alter the response of the pouch or of the stomach to histamine (dogs. 1 and 5, expt. d).

C. *The effect of oil of peppermint in the stomach upon Pavlov pouch acid secretion.* In 2 out of 4 experiments (expts. 3a and 4), in which oil of peppermint was given into the stomach and secretion stimulated by histamine, there was some depression of pouch secretion. In experiment 4 the amount of oil of peppermint employed was considerable (4 cc.). Larger quantities (8 cc.) had been utilized for pouches because here the oil drained off after five minutes, but in the stomach the oil necessarily remained. In experiment 3c no histamine was given, and there was no change in the basal secretion of the pouch.

D. *The influence of olive oil instillations into the pouch upon Pavlov pouch acid secretion.* In dog 3, olive oil in the pouch slightly diminished free acidity. Dog 4 presented a considerable increase in free acidity of pouch secretion after histamine stimulation. Fatty acids may explain the latter result.

E. *Olive oil in the main stomach.* One test was done. There was no change in the acid response of the pouch to histamine.

Discussion. Fourteen experiments (table 1, A) were performed upon five dogs in which, following the introduction of oil of peppermint into either pouch or stomach for five minutes, a subcutaneous injection of histamine was given. Of these fourteen determinations, twelve presented a reduction of more than 33 per cent in the free acidity of the stomach or pouch secretion, and two demonstrated no change. In one instance oil of peppermint in the pouch almost completely suppressed the free and combined acidity of a secretion following ingestion of food. Changes in volume and combined acidity were not constant, although frequently they were considerable.

Oil of peppermint in the jejunum (table 1, B) did not have an effect upon basal secretion, nor did it alter a histamine stimulated secretion.

In two out of four experiments, oil of peppermint in the stomach diminished the free acidity of a pouch after subcutaneous injection of histamine (table 1, C), but in one case the dose of oil was probably excessive (4 cc.).

TABLE 1

Summary of results

DOG NO.	POUCH	EXPT. NO.	HISTAMINE	OIL OF PEPPERMINT	± PER CENT CHANGE IN AREA OF SECRETION		
					Volume	Free acid	Combined acid
A. Oil of peppermint in pouch (secretion of pouch)							
			mgm.	*cc.*			
1	Heidenhain and jejunal cannula	a	0.125	8	0	−100	0
		b	0.25	6	0	−36	0
		c	0.25	6	0	−100	0
2	Pavlov	a	0.5	6	0	0	0
		b	0.25	6	0	−70	+82
		c	0.25	8	0	−100	+71
		d	0.25	7	0	0	+83
		e	Feeding*	6	0	−100	−78
3	Pavlov	a	1.0	5	−34	−40	−41
		b	0.5	6	−51	−92	0
		c	0.5	4	−62	−100	−53
		d	0.5†	5	0	−65	0
4	Pavlov		2.0	8	+78	−100	(+)
5*	Gastrostomy and duodenal cannula	a	1.0	30		−100	
		b	1.0	5		−100	
B. Oil of peppermint in jejunum (secretion of pouch in dog 1 and of stomach in dog 5)							
1	Heidenhain and jejunal cannula		0.25	2	0	0	0
5*	Gastrostomy and duodenal cannula	a		5		0	
		b		3		0	
		c		3		0	
		d		3		0	
		e	1.0	3		0	
C. Oil of peppermint in main stomach (secretion of pouch)							
2	Pavlov		0.5	2	0	0	+38
3	Pavlov	a	0.5	2	0	−54	−41
		b	0.5	2	0	0	0
		c		2	0	0	0
4	Pavlov		2.0	4	−36	−62	0
D. Olive oil in pouch (secretion of pouch)							
				OLIVE OIL *cc.*			
3	Pavlov		0.5	7	0	−41	0
4	Pavlov		2.0	6	+58	+137	0
E. Olive oil in main stomach (secretion of pouch)							
4	Pavlov		2.0	6	+48	0	0

* Results given as per cent changes of volume and acidity.

† Thirty minutes before oil of peppermint.

Histamine subcutaneously and olive oil in the pouch or main stomach did not produce consistent results in the secretion of the pouch (table 1, D and E).

The above experiments demonstrate that the most pronounced effect of oil of peppermint is a local one upon the pouch mucosa, counteracting the stimulation of acid secretion by histamine.

This diminution in free acidity cannot have resulted from an elevation in buffer content, for in the 11 experiments (table 1, A) presenting a depression in free acidity, the combined acidity remained constant in six determinations, and was decreased in three others. In only two tests (dog 2, b and c) did a rise of combined acidity occur which might suggest that buffers had neutralized the free acidity and in none of the specimens was blood found. This rise of combined acid may result from irritation of the pouch by the rubber tube inserted into it for introduction of the oil of peppermint.

These laboratory results are compatible with the clinical observation that the gastric secretory response of ulcer patients to both alcohol and histamine is usually greatly inhibited by the presence of oil of peppermint in the stomach. Moreover, the basal hyperchlorhydria of duodenal ulcer patients can also be decreased by 2 cc. of oil of peppermint; and it is significant that only in a few instances was the change between free and combined acidity in inverse ratio. The typical ulcer case, in whom oil of peppermint lessened the free acid titre of the gastric secretion stimulated by alcohol, exhibited neither an increase in visible mucus nor a rise in combined acidity, and therefore visible mucus and other buffers are to be dismissed as factors in the diminution of acidity. In a number of tests on patients in whom free acidity was reduced and combined acidity either unchanged or also reduced chloride determinations showed that the total Cl content remained unchanged (1).

Consequently the conclusion seems justified that oil of peppermint depresses gastric acid secretion, and usually without a concomitant increased production of buffer substances. The mechanism in all probability is a local one. At least a vagal reflex can be excluded for the response of Heidenhain and Pavlov pouches were comparable and parallel.

SUMMARY

The effects of oil of peppermint upon gastric secretion were investigated in five dogs with Pavlov and Heidenhain pouches, gastrostomies or enterostomies.

Oil of peppermint applied locally to secreting gastric mucosa causes a suppression in free acid flow, which is not associated with a relative increase in buffer activity.

We wish to thank S. Drexler and A. Pilon for their valuable technical assistance.

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SEPARATION OF A THYROTROPIC FROM THE GONADOTROPIC SUBSTANCES OF THE PITUITARY[1]

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At the present time considerable effort is being directed toward an elucidation of the exact functional relationship existing between the anterior lobe of the hypophysis and the thyroid gland. That the former exerts a definite excitatory influence over the latter has been adequately demonstrated by Allen (1916), Smith (1916), Uhlenhuth and Schwartzbach (1928), Loeb and Basset (1929) and others.

MATERIAL AND METHODS. In this investigation immature guinea pigs of both sexes weighing between 170 and 210 grams have been used as test animals for detecting the thyrotropic activity of various pituitary preparations. These guinea pigs were all of healthy stock and were housed in a room which was maintained at a constant temperature. Their daily diet consisted of a well balanced dry ration with small amounts of lettuce.

With the exception of phyone all preparations used were prepared in this laboratory. The methods of extraction outlined by Fevold and Hisaw for the preparation of gonadotropic substances served in large measure for extracting the thyrotropic material simply because these methods yielded the latter in abundance, especially in the earlier steps. As a consequence, the opportunity presented itself for studying the behavior of these substances in relation to each other. Furthermore, it is commonly agreed that the greatest difficulty in establishing the presence of a specific thyroid activating substance in the anterior pituitary has been to separate it chemically from the gonadotropic hormones of pituitary origin.

In following the course of the thyrotropic activity through various extractive procedures only histological evidence was used as a criterion of increased thyroid activity. It is a foregone conclusion that the physiological symptoms of hyperthyroidism which invariably accompany an initially induced hyperplasia of the thyroid are directly due to an increased outpouring of thyroid secretion.

Thyroid glands in different species vary in their response to pituitary stimulation. The rat and guinea pig are two rather well known extremes,

[1] Aided in part by a grant from the National Research Council, Committee on Problems of Sex, administered by Frederick L. Hisaw.

the rat being difficult to stimulate while the guinea pig is remarkably sensitive to pituitary therapy. Objection has been raised to the use of normal guinea pigs for studying thyroid behavior due to the great amount of variation said to be found in untreated animals. Approximately two hundred fifty guinea pigs have been used in this investigation and in no case has a hyperplastic condition ever been found in a normal animal or produced by toxic extracts, severe infections or excessive exploratory operative trauma which is in any way comparable to the hyperplasia characteristic of pituitary stimulation. It has been observed that the epithelium of the acini in the central portion of the gland is subject to a greater variation and is usually higher than that found in the acini near the periphery. In order to eliminate this slight variability only those sections which present a complete cross section through the center of the gland have been used in the diagnosis of a positive response.

The degree of thyroid response has been arbitrarily divided into three classes which have been indicated by one, two or three positive signs. The plus reaction indicates a low columnar epithelium with considerable liquefaction of colloid. The two plus reaction indicates a good response with a high columnar epithelium and almost total loss of colloid. The three plus has been reserved to indicate extreme cases of hyperplasia in which the cells of the larger acini are thrown into numerous papillary folds and in smaller acini the lumen is commonly obliterated by the tall epithelial cells. Colloid is of course either entirely absent or at the most represented by small amounts of foamy material.

The peculiarities and lack of information concerning the response of the guinea pig ovary to anterior pituitary therapy did not warrant using this species for the assay of gonadotropic preparations. Likewise, the greater resistance of the normal rat thyroid rendered this species impractical as a test animal for the thyrotropic substance. All gonadotropic materials were assayed on 21 day old female albino rats.

Chemical separation of the thyrotropic from the gonadotropic substances. During extraction the chemical behavior of the thyrotropic substance and crude gonad-stimulating extracts are for the most part identical. They are removed from the fresh gland or pituitary powder with the same extractives; are soluble in dilute aqueous alcohol, alkalies, acids or acetone and insoluble in concentrated organic solvents, and are both thermolabile to about the same degree.

Since the publication of a preliminary report by the author (1933) in which the association of the thyrotropic substance with the luteinizing fraction was noted, certain changes have been made in the method of fractionating the luteinizing substance from the one which promotes only follicular development which have had an important bearing on the present investigation. In the older method 60 per cent alcohol, barium oxide and

benzoic acid were used as precipitants for the fractional separation of the two gonadotropic substances. It has since been found by Fevold and Hisaw that the fractionation can be facilitated by iso-electrically precipitating the crude pyridine extract at a pH 4.2. At that point a heavy precipitate forms which is centrifuged off. The follicle-stimulating fraction remains in solution and with repeated precipitation is obtained in a highly purified form as judged by assay on immature female rats and rabbits. The heavy iso-electric precipitate is repeatedly extracted with buffer solution at pH 8.0. The supernatant solutions are again adjusted to pH 4.2 and the precipitate is thoroughly washed with acetone and dried. The luteinizing factor is contained in this powder. Employing the above method it was found that the thyrotropic material was no longer associated

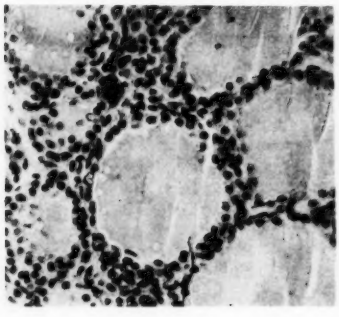

Fig. 1

Fig. 2

Fig. 1. Thyroid of a guinea pig which had been treated five days with the benzoic acid soluble fraction in the equivalent of 1.5 gram sheep pituitary. × 320.

Fig. 2. Thyroid of a guinea pig which had been treated five days with the benzoic acid insoluble fraction in the equivalent of 1.5 gram sheep pituitary powder. × 320.

with the luteinizing fraction but occurred always in the iso-electric soluble or follicle-stimulating fraction. This complete shift in the occurrence of the thyrotropic substance from the luteinizing fraction by one method of extraction to the follicle-stimulating by an entirely different method strongly suggested that the thyroid effect was being produced by a pituitary influence that was clearly not identical with either of the gonadotropic substances.

The thyrotropic activity can be separated from the contained gonadotropic substances (table 1) by precipitating the isoelectric soluble fraction referred to above with benzoic acid (figs. 1 and 2). The thyroid-stimulating substance is precipitated quantitatively. The benzoic acid soluble and insoluble fractions and a sample of the original iso-electric soluble extract were each tested on immature rats at the same dosage level (0.25

gm. pituitary powder equivalent). The benzoic acid soluble fraction produced practically the same increase in ovarian weight (500 per cent) as the original extract. The insoluble material, however, did not open the

TABLE 1

The thyrotropic substance can be precipitated with benzoic acid from an iso-electrically soluble pituitary preparation leaving the gonadotropic substances in solution

(All animals autopsied after five days' treatment)

SOLUTION	GUINEA PIG				RAT	
	GP.	Dose*	Thyroid, weight	Hyperplasia	Dose*	Ovaries, weight
			mgm.			*mgm.*
Iso-elect. pH 4.2 soluble	199	1	35	+++	0.25	70
	167	1	60	+++	0.25	75
	198	1	34	++		
	168	1	65	+++		
	200	1	40	+++		
	192	1	56	+++		
	153	1	49	+++		
The above boiled	205	1	18	−	0.25	10
	206	1	14	−	0.25	11
(Uninjected controls)	81		29	−		
	228		25	−		
Benz. acid sol. fract.	213	1	26	−	0.25	54
	216	1.5	22	−	0.25	62
	219	1.5	28	−	0.25	58
	225	1	24	−		
Benz. acid insoluble	215	1.5	47	+++	0.25	13
	217	1.5	44	+++	0.25	17
	218	1.5	50	+++	0.25	11
	227	1	49	+++	0.25	11
Horse A.P. foll. stim. benz. acid sol. fract.	132	1	24	−	0.25	31
	194	0.5	18	−	0.25	36
	46	1	14	−		

* Dosages for both guinea pigs and rats are given as grams equivalent of pituitary powder.

vaginae of immature rats though it did occasionally produce a slight increase in ovarian weight (2–4 mgm.).

Effect of heat treatment. If we assume that the pituitary stimuli acting on the thyroid and gonads are distinct substances it is interesting to study

the effect of heating a preparation known to contain both of these properties. The general impression has been gained from the literature that both the gonadotropic and thyrotropic substances in water soluble form are destroyed by boiling, but the effects of lower temperatures have not been thoroughly investigated. In this study two preparations were used: one, the iso-electric pH 4.2 soluble fraction of dried sheep pituitary extract, possessing both gonadotropic and thyrotropic potency; the other a commercial product, phyone, which contains in addition to its growth promoting ability, thyrotropic (Schockaert, 1932) and gonadotropic (Hertz et al., 1932) properties. These preparations were subjected to temperatures ranging between 60 to 100°C. for fifteen minutes. In each case thyrotropic ability was assayed (table 2) on two immature guinea pigs and the gonadotropic (of only the iso-electric soluble fraction) on two 21 day old female rats. No deleterious effects were observable at 60°C. but both the gonadotropic and thyroid stimulating property were found to be seriously impaired at 70°. At 80° the thyrotropic material was completely inactive and the gonadotropic material was nearly so, while at 100° this too was entirely destroyed. The dosages were maintained constant for each of the two

TABLE 2

Showing the effects of heating a pituitary preparation (iso-electric pH 4.2 soluble fraction) possessing both thyrotropic and gonadotropic ability

Total dosage for each guinea pig was one gram equivalent pituitary powder and for each rat 0.25 gram. Duration of treatment in all cases was five days.

GUINEA PIG					RAT
Guinea pig	Temperature	Time heating	Thyroid, weight	Thyroid reaction	Ovaries, weight
	°C.	*minutes*	*mgm.*		*mgm.*
192	unheated		40	+++	36
200	unheated		58	+++	25
177	60	15	80	+++	25
178	60	15	70	+++	45
201	70	15	40	++	25
202	70	15	35	++	23
203	80	15	17	−	10
204	80	15	22	−	21
205	100	15	18	−	10
206	100	15	14	−	11
179	100	15	28	−	
180	100	15	26	−	

preparations and were of sufficient magnitude in each case to render a sharp contrast in the response to the heated and unheated material, especially at the higher temperatures.

Krichesky (1934) reported that boiling phyone for fifteen minutes destroyed its growth promoting and gonadotropic ability as tested on young mice and adolescent rabbits respectively, but did not seriously reduce its ability to stimulate the thyroids of tadpoles. On the basis of mammalian reactions obtained in this laboratory it is unequivocal that heat cannot be used as a means of inactivating the gonadotropic substances without also destroying the thyrotropic potency of an unpurified pituitary extract.

Effect of placental extract, urine and blood serum of pregnancy. Extracts of placenta, urine and blood serum of pregnancy have been reported to have no effect on thyroid structure (Kline, 1929; Loeb, 1932; Evans, 1933). It seemed desirable to test these preparations further by administering excessive quantities. The fifteen guinea pigs used in these tests were divided into 3 groups of 5 each. One group received 20 R.U.[2] each of human placental extract. In another group each animal received an extract of the urine of pregnant women equivalent to 400 cc. (270 R.U.). The third group received 120 R.U. each of an unfractionated pyridine extract of pregnant mare serum. The time of treatment in each case was 5 days. There was no evidence in any of these animals that any change from the normal thyroid had been induced by these extracts.

Effect of continued injections. The inability of the thyroid to maintain itself in a hyperfunctioning capacity in the presence of chronic hyperpituitarism has been well established (Schockaert, 1932; Collip, 1933; Friedgood, 1934). We wished to determine whether the thyrotropic material which has formed the basis of this report would induce a series of events similar to those reported by the above authors. A group of eight young guinea pigs were started on a thyrotropic preparation which had been previously standardized for thyroid stimulation. The dosage for each animal was one gram equivalent sheep pituitary powder per week. At weekly intervals two animals of this group were killed and the thyroids prepared for histological study. The maximum injection period was 28 days. The greatest amount of stimulation was found at one week. By the end of the third week the histological appearance of the thyroid was practically back to normal and at the end of the fourth week it was indistinguishable from normal glands. The weight curve of all these animals showed a more or less precipitious fall initially with a gradual return to the previous level in those receiving the injections over three and four weeks.

DISCUSSION. That the thyrotropic substance is not identical with the

[2] The rat unit as used here denotes ovarian stimulation in the 21 day old rat to the extent of effecting within five days definite follicular or luteal development and a weight increment of 50 to 100 per cent over control ovaries.

growth hormone was indicated by Smith (1930), Crew and Weisner (1930), Riddle et al. (1933) and Collip (1933). Evans et al. (1933), however, state that their growth promoting preparation has a reparative action on the thyroid in the hypophysectomised rat. It is clear that this preparation is contaminated with the thyrotropic substance. A careful examination of their extractive procedure for the growth preparation reveals no process that should effectively eliminate the thyrotropic substance.

The task of distinguishing the thyrotropic from the gonad-stimulating substances has been more difficult. The marked similarities of these two substances in chemical behavior has been noted. That they are different substances was indicated by the failure of gonadotropic preparations of extra-pituitary-sources to stimulate the thyroid. This, however, cannot be taken as conclusive evidence for the anterior-pituitary-like substances are not identical in every respect with those from the anterior pituitary.

In this report the primary objective has been to establish the presence in the hypophysis of a thyroid-stimulating factor distinct from the gonad-stimulating hormones. The fractionation of the active material for the two glands concerned was effected by precipitation with benzoic acid. The thyrotropic activity is confined to the precipitate. The degree of separation of the gonad-stimulating material between the benzoic acid soluble and insoluble fraction is in the neighborhood of 91 to 97 per cent as calculated on the basis of ovarian weight increments. It was expected that a trace of the gonad-stimulating material would be carried over by the benzoic acid treatment for the precipitate is heavy and offers chances of adsorption. This slight contamination of the thyrotropic material is of no significance in establishing the thesis of this report. The conception that the gonad-stimulating substances are responsible for thyroid stimulation is opposed to the experimental facts.

SUMMARY

The fractionation of a pituitary extract known to be active for both thyroid and gonad-stimulation was effected by precipitating the thyrotropic factor with benzoic acid. Heat cannot be used to destroy the gonad-stimulating activity in a crude pituitary preparation without also destroying the thyrotropic substance. Extracts of placenta, urine and blood serum of pregnancy have no effect upon the structure of the thyroid gland. The experimental evidence indicates that the thyroid-stimulating factor is a distinct pituitary derivative.

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THE NERVOUS CONTROL OF INTERCOSTAL RESPIRATION

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It is the purpose of this paper to present certain observations relating to the mechanism of intercostal respiration and to the activity of the motor nerve cells controlling the intercostal muscles. The experiments deal especially with the discharge from single nerve cells during normal respiration and asphyxia, the reciprocal relation of the two groups of cells supplying the muscles of inspiration and expiration, and the character of the discharge from these motor centers when they are uninfluenced by afferent impulses resulting from respiratory movements.

The importance of such impulses from the lungs in regulating the activity of the respiratory center has long been recognized and a recent paper by Adrian (1933) gives an excellent analysis of the afferent mechanism and its reflex effects. This reflex is indeed so striking that it is still argued by some that the rhythmic discharge of impulses from the respiratory center does not continue in the absence of periodic bursts of impulses from the lungs and the respiratory muscles. There is, however, considerable evidence that such spontaneous rhythms develop within the cells of the centers themselves when they are uninfluenced by the rhythmically recurring afferent impulses (Winterstein, 1911; Adrian, Bronk and Phillips, 1932; Adrian, 1933). It is with the behavior of these cells under such conditions and when played upon by the afferent impulses that these experiments are primarily concerned.

The experiments were performed on cats decerebrated or under nembutal anesthesia. After removal of the latissimus dorsi muscles small terminal twigs of the intercostal nerves were dissected out of the external and internal intercostal muscles, thereby definitely determining the final distribution of the nerve fibers under study. The nerve, still in connection with the main trunk of the intercostal nerve, was then placed on brush electrodes leading to the input of a resistance-capacity coupled amplifier, the output of which was connected to an oscillograph and loud speaker. In most of the experiments the nerves were split apart by means of fine needles until the discharge consisted of a single series of impulses in one

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fiber. Immobilization of the animal was effected by means of curare; when this was not done considerable care was necessary in order to avoid artifacts caused by movements of the nerve on the electrodes.

Figure 1 is a typical record of the impulses in a single motor nerve fiber to an external intercostal muscle during normal inspirations of moderate depth. The discharge is essentially the same as that which has been recorded in single fibers of the phrenic nerve (Adrian and Bronk, 1928). It consists of a succession of short bursts of impulses, the impulses within each burst coming with a fair degree of regularity at a frequency which increases as the contraction develops. Figure 2 is an excellent illustration of what variations in the nerve discharge effect variations in the magnitude of respiratory movements. Using the discharge in a small nerve to an external intercostal muscle as an index of the activity of the motor nerve cells regulating the respiration of the animal, we see that at A an inspiration of moderate depth is associated with a short volley of impulses in three fibers. The next inspiration at B was much deeper and here more nerve cells were in action, discharging at a higher average frequency and for a longer duration of time. Following this there was a very shallow inspiration associated with which there was but a single impulse in each of two fibers. Deeper inspiration is the result of an increased frequency of discharge from the individual motor nerve cells, more neurones are in action and they discharge for a longer period of time. It may be pointed out in passing that in the original work (Adrian and Bronk, 1928) on the discharge of impulses in single fibers of the phrenic nerve no evidence was found for a grading of the diaphragmatic contractions by means of a variation in the number of active neurones. In the case of the intercostals, however, this mechanism plays an important rôle as was found to be the case in reflex or voluntary contractions (Adrian and Bronk, 1929).

The impulses are not always grouped into bursts as described above. Sometimes, as in figure 3, there is a continuous train of impulses whose frequency rises and falls with the respiratory cycle. The muscle fibers supplied by these nerve fibers would therefore be in a continuous state of contraction, the degree of which would vary during inspiration and expiration.

A study of the function of the nerve fibers to the external and to the internal intercostal muscles has shown that generally the discharge of impulses to the former is during inspiration and to the latter during expiration as in figure 4. In a decerebrate cat breathing quietly, however, there were often no such impulses and the discharge only developed after adding some degree of respiratory obstruction. With the onset of partial asphyxia bursts of impulses began first in the nerves to the external and later in the nerves to the internal intercostal muscles. It should also be pointed out that in some cases the impulses in nerve fibers supplying the

Fig. 1. Impulses in a single motor nerve fiber to an external intercostal muscle. Time marker: $\frac{1}{5}$ second. Lower line: pneumogram, upward movement indicating inspiration.

Fig. 2. The discharge of motor impulses in a nerve twig to an external intercostal muscle in three successive inspirations of varying depth. A, moderate; B, deep; C, shallow. Time and pneumogram as in figure 1.

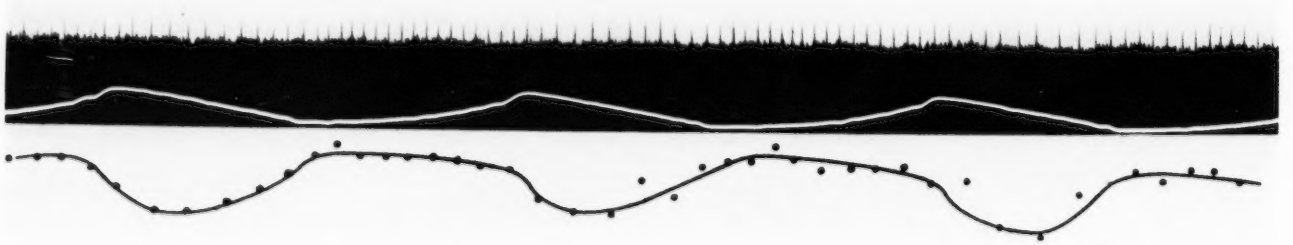

Fig. 3. Variations with respiration in the frequency of impulses to an external intercostal muscle from a motor nerve cell in continuous activity. Pneumogram as in figure 1. Lowest line represents the impulse frequency.

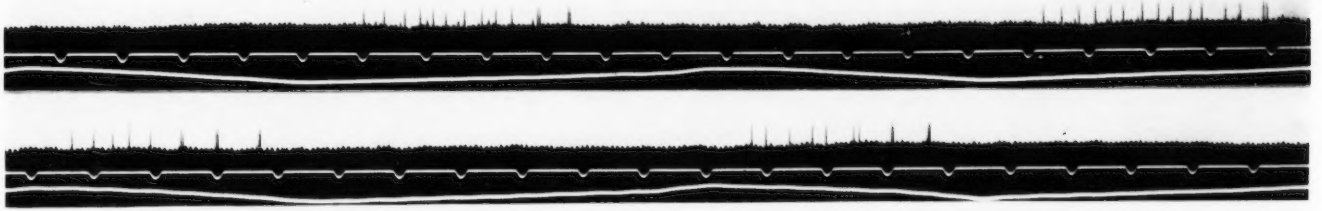

Fig. 4. The discharge of motor nerve impulses to, above, an external intercostal muscle and, below, an internal intercostal muscle. Time in $\frac{1}{5}$ second. Upward movement of pneumogram indicates inspiration.

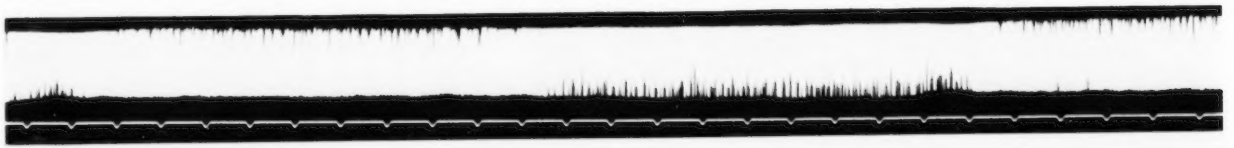

Fig. 5. Simultaneous records of motor nerve impulses to an internal intercostal muscle (upper record) and to an external intercostal muscle (lower record), vagi and carotid sinus nerves cut, animal completely immobilized with curare.

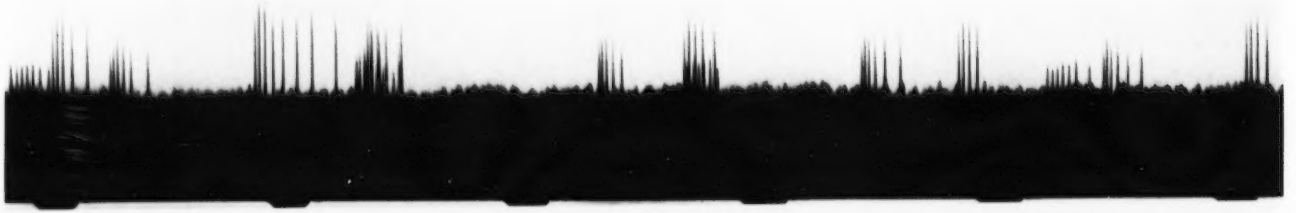

Fig. 8. Grouped discharges from three motor nerve cells supplying an intercostal muscle during asphyxial failure

internal intercostal muscles were found to be synchronous with those to the external intercostals and therefore with inspiration. Such a simultaneous action of the two sets of muscles might tend to splint the thorax for the more effective action of the diaphragm. The usual picture which we have observed, however, is a reciprocal alternating discharge of nerve impulses to the external intercostal muscles during inspiration and to the internal intercostal muscles during expiration. This statement applies to the intercostal parts of the muscle. If, on the other hand, fibers are dissected out of the interchondral part of the internal intercostal muscle, one finds that the impulse discharge occurs during inspiration, a reversal of function which conforms with the difference in the anatomical relation of the fibers of this portion of the muscle to the axis of the ribs. These observations are in agreement with generally accepted opinions as to the function of these muscles. They are recorded here because one finds from time to time contrary statements regarding their action, and because they provide such an excellent picture of nerve impulses effecting reciprocal muscular action.

We were especially interested in how this reciprocal relationship of the nerve cells supplying these two sets of muscles would be affected by the removal of periodic bursts of afferent impulses caused by movements of respiration. In order to eliminate those from the tension receptors in the lungs, the mechanism of which has been described so admirably by Adrian (1933), both vagi were sectioned in the neck. Such a procedure would however, as Schafer (1932) has pointed out, leave rhythmic afferent impulses from the respiratory muscles. The animal was therefore completely immobilized by the intravenous injection of curare. A nerve twig supplying an external intercostal muscle was then placed on a pair of electrodes connected to one amplifier and oscillograph, and a nerve supplying an internal intercostal muscle on electrodes connected to a second amplifier and oscillograph. In this way simultaneous records were obtained of the discharges from the two sets of nerve cells. During over-ventilation by means of a respiration pump no impulses were observed in either nerve. When ventilation was stopped and asphyxia ensued, rhythmic bursts of impulses developed in the two nerves, first in that to the external intercostal and later in that to the internal. As a rule the bursts alternated and when, as was frequently the case, there were long continued discharges to the external intercostals this discharge abruptly ceased at the beginning of the discharge to the internal intercostal, and began again as this second burst stopped. Such alternating discharges are shown in figure 5. Briefly then, there is a rhythmic activity of the nerve cells regulating respiratory movements of the intercostal muscles in the absence of periodic groups of afferent impulses, and furthermore, the two groups of cells controlling the expiratory and inspiratory muscles continue to maintain their recip-

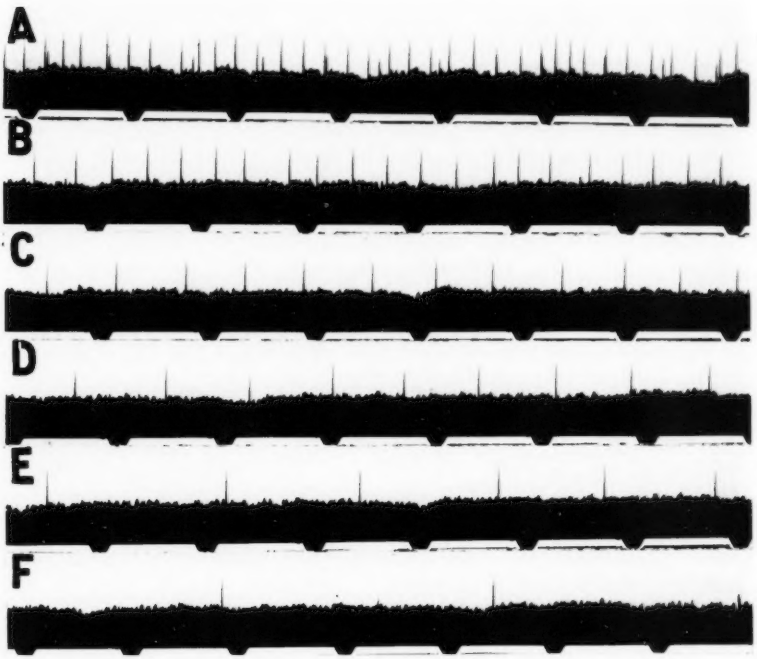

Fig. 6. Response of a motor nerve cell supplying an internal intercostal muscle to decreasing degrees of asphyxial stimulation. Cat curarized. Respiration pump started at A after prolonged asphyxia. Records at 10 second intervals. Time marker: $\frac{1}{5}$ second.

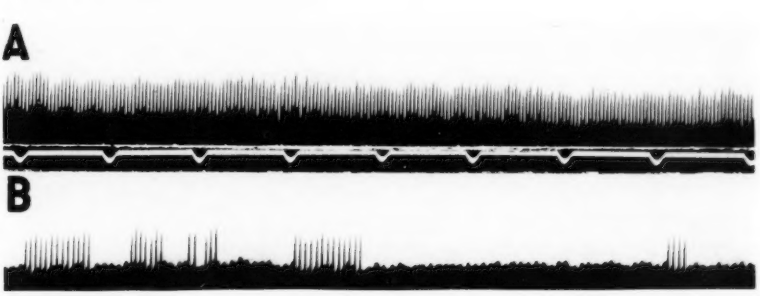

Fig. 7. A. Motor impulses to an internal intercostal muscle in late stages of asphyxia. B. Failure of the nerve cell somewhat later.

rocal relationship in the absence of those impulses. This is in agreement with Adrian's findings relating to the reflex significance of afferent impulses from the lungs. We find that the two sets of nerve cells do not as a rule discharge simultaneously, development of activity in one group being coincidental with a cessation of activity in the other. However, if asphyxia be prolonged beyond a certain stage this is no longer true. The discharge then becomes continuous and the two sets of cells may discharge simultaneously. The reciprocal control is broken down but recovers if the animal is again ventilated.

The response of a single nerve cell supplying a respiratory muscle to varying degrees of stimulation has been studied in the following manner: In a curarized animal, with vagi and carotid sinus nerves sectioned, an intercostal nerve twig containing several active fibers was placed upon the electrodes and the discharge observed during the development of asphyxia. This was induced by stopping the respiration pump. In figure 6 at A the frequency of impulses in one fiber was about 25 per second, in another 9 per second. At this instant the respiration pump was again started and records B, C, D, E and F were taken at 10 second intervals during the recovery from asphyxia. The second fiber promptly dropped out of action and the frequency of discharge in the first decreased in the successive 10 second periods to 16, 8, 6, 4 and 2 per second. Here is an excellent illustration of the chemical control of the nerve cells regulating respiration. The progressive decrease in frequency of discharge from a motor nerve cell with a decrease in the degree of asphyxia is strikingly similar to the decrease in frequency of impulses from a sense organ with a lowered intensity of stimulus.

If the asphyxia is continued to the terminal stage the impulse frequency rises to a high value. Subsequently the cell is unable to maintain such a rate of activity continuously as is shown by the fact that whole groups of impulses begin dropping out of the regular series (fig. 7). The gaps become more numerous and longer in duration with a resulting irregular type of discharge. Sometimes this consists of random spaced groups of impulses, the impulses in any one burst starting at a high frequency which quickly drops to a lower level at the end of the burst. Such discharges from three motor nerve cells are shown in figure 8. Finally all activity ceases. This is the general picture which we have observed of the irreversible failure of a motor nerve cell as a result of asphyxia.

It is interesting to notice that the transition from a regular to an irregular discharge shown above is very much the same as that observed when a muscle spindle fails as a result of fatigue (Bronk, 1929). There too "the failure of response is characterized by one or more impulses dropping out of an otherwise fairly regular series, the impulses becoming more and more scattered until finally the discharge ceases." A like type of failure

has also been observed by Adrian (1930) in injured mammalian nerves, and furthermore he finds there under certain conditions a type of grouped discharge very similar to that shown in figure 8. It differs in that the groups are periodic rather than irregular but the impulses in each group gradually decrease in frequency to the end of the burst. Such analogies in the behavior of motor nerve cells, sense organs and the nerve fiber itself emphasize again the essential similarity of the mechanisms involved.

SUMMARY

The discharge of impulses from single motor nerve cells to the external and internal intercostal muscles of a cat has been recorded.

1. Each contraction is the result of a short burst of impulses. Variations in depth of intercostal respiration are a result of variations in the frequency of discharge from the individual nerve cells, the duration of their discharge and the number of nerve cells in action.

2. The rhythmic groups of impulses in the fibers to the external intercostal muscles are generally associated with inspiration; in the fibers to the internal intercostal muscles with expiration. The discharge in the interchondral part of the internal intercostal muscle is, however, synchronous with inspiration.

3. This rhythmic discharge of motor impulses continues when the volleys of afferent impulses resulting from respiratory movements are eliminated. The normal reciprocal character of the activity in the two sets of cells supplying the external and internal intercostal muscles is likewise maintained in the absence of the rhythmic afferent volleys.

4. The relation between the frequency of discharge from the individual motor nerve cells and the intensity of stimulus has been observed by varying the degree of asphyxia. The impulse frequency increases with increasing asphyxia.

5. During respiratory failure of an animal the frequency of impulses from these cells increases to a high value. The discharge finally fails as the result of more and more impulses dropping out of the regular rhythm which thus becomes a series of scattered volleys.

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THE RESPONSE TO STEADY PRESSURES OF SINGLE END ORGANS IN THE ISOLATED CAROTID SINUS

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In a previous paper (Bronk and Stella, 1932) we have described the nature of the nervous discharge from single pressure receptors in the carotid sinus. It was shown that there is a volley of afferent impulses with each arterial pulse, the frequency of impulses in each volley and the duration of the volley depending on the mean blood pressure, the form of the pulse curve and the characteristics of the individual receptor. At pressures below 40 to 50 mm. Hg there is usually no discharge of impulses from the end organ; as the pressure is increased there are one or a few impulses at the height of systole and at higher pressures the fiber conducts a train of impulses from the end organ to the cardio-vascular centers continuously throughout the heart cycle. Furthermore, at higher pressures there are more end organs in action. As the result of increased arterial pressure therefore the centers receive a larger number of impulses per unit of time. This is the quantitative basis for the afferent side of the cardio-vascular reflexes arising in the carotid sinus.

The present investigation is devoted to a study of certain of the properties of the pressure receptors which give rise to this afferent discharge; and especially to the way in which they respond to continuous and changing pressures. By perfusing the isolated sinus it has been possible to hold the pressure at a constant level for any desired time or to vary it at will in a manner which is not possible when the receptors are stimulated naturally by the arterial pulse. This more adequately controlled stimulus has thus enabled us to secure information of value in explaining some of our previous observations and has added to our knowledge of the properties of sense organs in general and especially those which play so important a rôle in the regulation of blood pressure.

Rabbits anesthetized with urethane were used for the experiments. All of the arteries leading to or from the carotid sinus were ligated excepting the common carotid and one of the larger branches of the external carotid. Into these two were tied cannulae which led to a reservoir of defibrinated blood. In this system were a perfusion pump, a valve for producing unidirectional flow and a side connection to a mercury manometer. The

nerve from the sinus was dissected out, cut at its junction with the glossopharyngeal and all but one or a few of the active fibers were transected close to the sinus. Thus there was a lead from the main trunk of the nerve on which the electrodes were placed to only one active fiber and it was therefore possible to observe the discharge from a single sense organ.

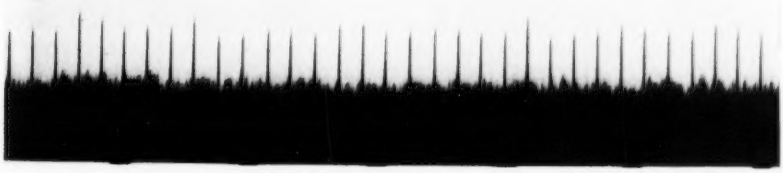

Fig. 1. Afferent impulses from a single end organ in the carotid sinus when stimulated by a constant pressure within the sinus. Time marker gives $\frac{1}{5}$ second.

The electrodes were connected to a resistance-capacity coupled amplifier the output of which supplied a Matthews oscillograph and a loud speaker. The sensitivity of the recording system was such that ten microvolts applied to the input of the amplifier gave an oscillograph deflection of about 10 mm.

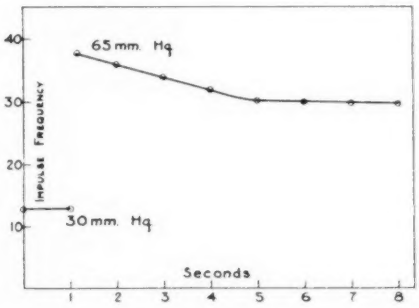

Fig. 2

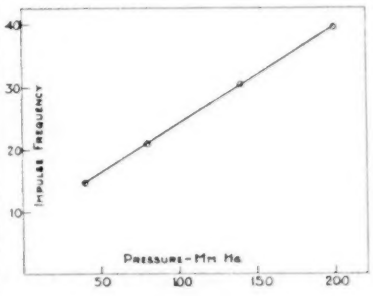

Fig. 4

Fig. 2. The decline in frequency of afferent impulses from a single end organ in the carotid sinus stimulated by a constant pressure of 65 mm. Hg.

Fig. 4. Relation between the frequency of discharge from a pressure receptor in the carotid sinus and the endosinusal pressure.

Response to constant pressure. When a receptor is stimulated by a constant pressure there is, as shown in figure 1, a discharge of impulses which follow one another at a fairly regular frequency, a discharge which is characteristic of most sense organs when subjected to a constant stimulus (Adrian, 1932). The duration of such a response to a continued stimulus

varies considerably, however, with different types of receptors: in the case of those which adapt rapidly the frequency of impulses falls quickly and the duration of the discharge may be extremely brief, as in the case of touch receptors (Adrian, Cattell and Hoagland, 1931). Others, such as the muscle spindle or tension receptors in the lungs (Adrian and Zotterman, 1926; Adrian, 1933) continue to discharge impulses throughout the course of a long continued stimulation and at a frequency that falls comparatively little from its maximum value. In our former paper we assumed on the basis of indirect evidence that the pressure receptors in the carotid sinus belonged in the latter category. Our present results show that this is definitely the case. Figure 2, for instance, gives the frequency from a single receptor when stimulated by a pressure of 65 mm. Hg. During the first 6 seconds while reaching a fairly steady adaptation level there was a decrease in frequency of only about 25 per cent. Such values indicate that these receptors are of a very slowly adapting type which is of interest in the following connection. A receptor for which the normal stimulus is pulsatile and intermittent could have a rapid rate of adaptation and be repeatedly stimulated by each successive pulse. But if associated with higher pressures there were a smoothing out of the pulse pressure, the frequency of the impulses would not rise to as high a level. A slowly adapting end organ would seem therefore to be more effective for accurately reporting the level of mean blood pressure.

Response to various pressures. Because these end organs are important agents in preventing wide fluctuations in blood pressure their response to different pressures is of prime importance. We have previously shown that the average frequency of the afferent impulses increases with the mean pressure, but the pulsatile nature of the stimulus made it impossible to obtain quantitative information regarding the relationship between these two variables. An excellent series of records obtained by the present technique at constant pressures ranging from 40 mm. Hg to 200 mm. Hg is shown in figure 3. The frequency of impulses at these four pressures is plotted in figure 4 and it will there be observed that this range of pressures has produced a two and one-half fold increase in frequency. Although such a striking linear relationship over the entire range of pressures investigated has been found in many experiments, in others the frequency does not increase after the pressure has reached a level of from 140 to 180 mm. Hg with a maximum variation in frequency coming between limits within which one would expect the carotid sinus reflex mainly to function. It must be emphasized that there are wide variations in the impulse frequencies from different individual end organs and in their respective thresholds.

Such differences in threshold provide the basis for further increasing the range of the signalling mechanism. Figure 5, for instance, shows the

discharge from two end organs, A when the carotid sinus was distended by a pressure of 70 mm. Hg and, B by a pressure of 100 mm. Hg. The lower pressure was adequate to stimulate only one of the receptors con-

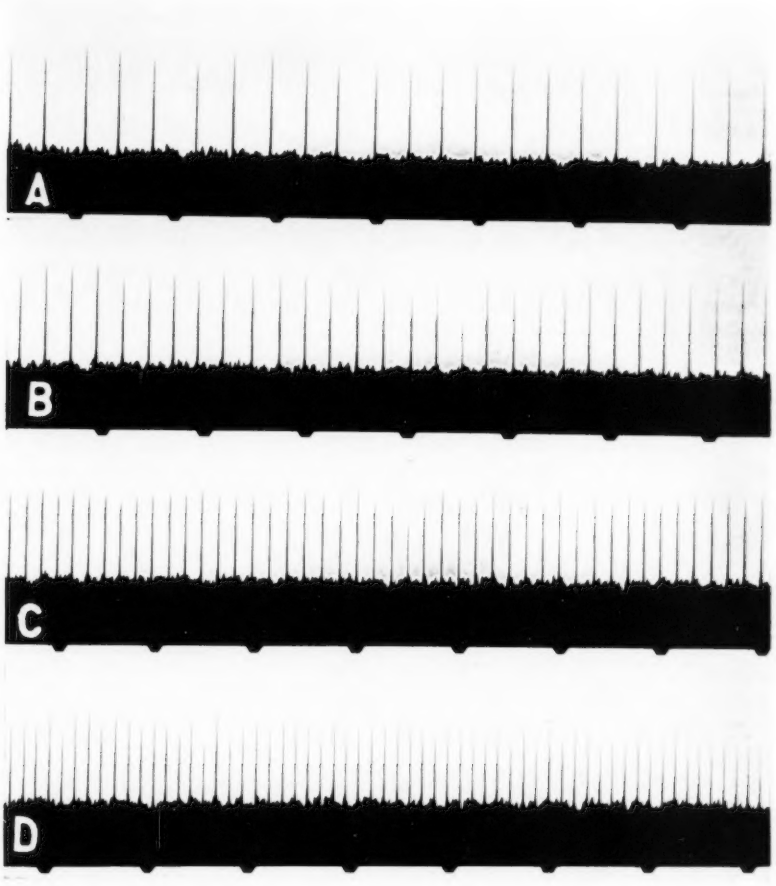

Fig. 3. Afferent impulses from a single end organ in the carotid sinus stimulated by constant pressures within the sinus. In A: 40 mm. Hg; B: 80 mm. Hg; C: 140 mm. Hg; D: 200 mm. Hg. Time marker gives $\frac{1}{5}$ second.

cerned. When the pressure was raised to the higher value the frequency of the impulses from this first receptor increased from 15 to 25 and a second receptor came into action discharging at a rate of 20 per second.

Thus a rise in pressure increases the number of afferent impulses from the sinus by increasing both the frequency of discharge from the individual end organ and by increasing the number of active end organs.

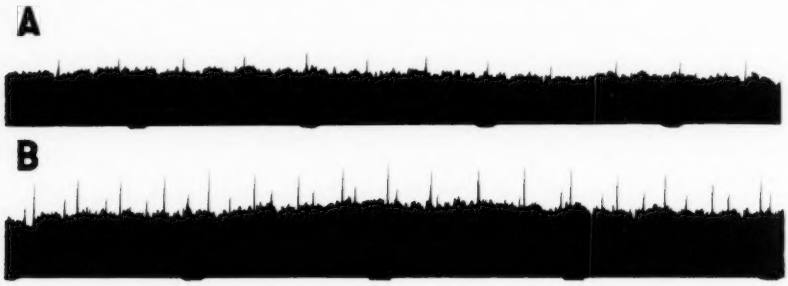

Fig. 5. Discharge of impulses from two end organs, A, when stimulated by a pressure of 70 mm. Hg, and B, 100 mm. Hg. The second end organ comes into action in B.

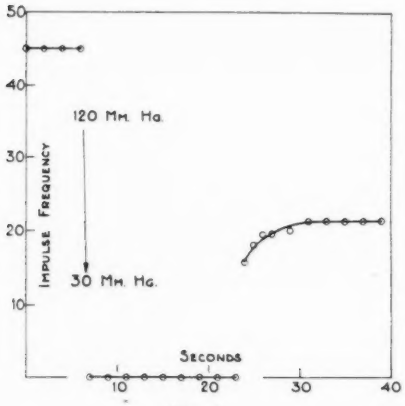

Fig. 6

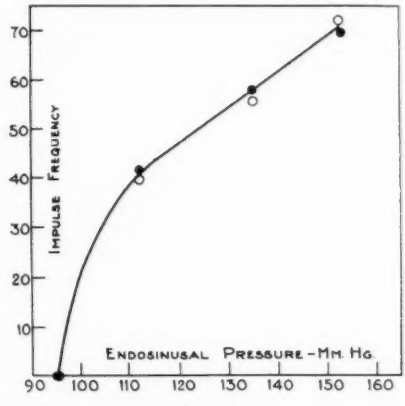

Fig. 7

Fig. 6. The temporarily complete cessation of discharge from a carotid sinus receptor following a decrease in pressure from 120 mm. Hg to 30 mm. Hg and its subsequent return.

Fig. 7. The response of a pressure receptor to various pressures, a, (full circles), with the sinus perfused with normal arterial blood and, b, (open circles), with blood from the animal after a prolonged period of rebreathing N_2.

Response to a sudden drop in pressure. When the sinus is subjected to the normal stimulus of the arterial pulse one finds that at low or medium pressures there are impulses only during systole even though the diastolic pressure is of a value sufficient to evoke a discharge if maintained continuously. Because of that observation we were interested in studying

the effect of suddenly lowering the pressure from one constant level to another.

Results of such experiments are shown in figure 6. When the pressure within the sinus was quickly dropped from 120 mm. Hg to 30 mm. Hg the impulse frequency did not fall from 45 per second to that characteristic of the lower pressure, but the discharge stopped completely for a period of about 18 seconds and then gradually developed to its new level. This evidently accounts for much of the decrease in discharge from the carotid sinus with the rapid diastolic drop in pressure. It may also be pointed out that this silent period accompanying a decrease in stimulus intensity is similar to an effect of much shorter duration found in the case of muscle spindles by Bronk (1929) and Matthews (1931).

Effect of changes in the chemical composition of the blood. It has been shown by Heymans (1933), Schmidt (1932a, b), and others that marked respiratory reflexes are elicited by perfusing the carotid sinus with blood low in O_2 content. If the receptors sensitive to variation in pressure are those responsible for this respiratory reflex their response should be materially modified by variations in the chemical composition of the blood in the sinus.

It was difficult to answer this question satisfactorily in our previous paper because asphyxia or inhalation of CO_2 produces variations in blood pressure as well as changes in the composition of the blood. With the isolated and perfused sinus it has however been possible by the following procedure in a heparinized animal. With normal arterial blood in the sinus the flow was stopped, the pressure within the sinus was established at a certain level and the frequency of impulses from an end organ was recorded. The pressure was then raised to a higher level and the impulse frequency again noted. This was repeated for a series of pressures after which the sinus was again perfused with blood from the systemic circulation. The animal was then given a mixture high in N_2 and low in O_2 to rebreathe until the blood became highly venous in appearance. With this blood in the sinus the above procedure was again followed. The results of a typical experiment are shown in figure 7. It will be seen that the response to the pressure stimulus is in no way modified by such a marked change in the composition of the blood. Similar results were obtained with mixtures high in CO_2. The pressure receptors in the carotid sinus are evidently highly insensitive to changes in the chemical composition of their environment.

This should not be taken as evidence against the validity of the findings of Heymans and Schmidt that the carotid sinus has an important rôle in the reflex regulation of respiration. It rather indicates that the pressure receptors, at least those which we have studied, do not respond in a measurable degree to changes in the chemical composition of the blood which are within physiological limits. It favors the theory that there are

two types of receptors in the region of the carotid sinus—one for pressure reflexes and one for chemical. This agrees with the view put forward by Schmidt (1932a).

The fact that these receptors are insensitive to changes in the composition of the blood is a further illustration of their remarkable capacity for providing the centers with accurate information regarding the level of pressure within the arterial system. Were their response appreciably modified by chemical changes in the blood the number of afferent impulses going to the centers would not be a faithful index of the level of arterial pressure.

SUMMARY

The properties of the pressure receptors in the carotid sinus have been investigated by isolating and perfusing the sinus and recording the impulses from single end organs.

1. The response of such an end organ to a steady pressure consists of a train of impulses following one another with a high degree of regularity.

2. This discharge continues indefinitely at a frequency but slightly less than the initial maximum value. Thus these receptors are of the type that adapts but little.

3. The frequency of response increases with increased pressure in the sinus.

4. There is a wide variation in threshold for different receptors. By means of the above two factors variations in pressure give rise to variations in the number of afferent impulses from the sinus per unit of time.

5. Associated with a drop in pressure within the sinus there is a complete cessation of impulses from the end organ, which after some seconds begins discharging again at a frequency characteristic of the new pressure level. The relation of this phenomenon to the production of a pulsatile type of discharge accompanying the arterial pulse is discussed.

6. These pressure receptors are insensitive to variations in the CO_2 and O_2 content of the blood. This indicates that the respiratory reflexes arising within the carotid sinus are due to a different set of end organs.

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